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THE ACTIVE PRINCIPLES OF DIFFERENT ORGANS, AS SHOWN IN KYMOGRAPH TRACINGS[1]

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A recent report from the Department of Experimental Therapeutics in the Cornell University Medical College, described the effects produced by the intravenous injection in dogs of certain proteids and extractive materials which can be isolated from an aqueous extract of the thyroid gland.[2]

The nucleoproteins, globulins and coagulable proteins are each found to be entirely without influence upon the kymograph tracings except in massive or overwhelming dosage. This was contrary to what one of us had expected because when the nucleoproteins and globulins are administered to patients who present symptoms of thyroid disease, these substances seem very active in either relieving or intensifying the preëxisting disturbance.

After the removal of the nucleoproteins, globulins and coagulable proteins, the filtrate which remains was found to produce a marked fall in blood pressure, a slight acceleration of the pulse rate, and a slight deepening of respiration. This filtrate was

[1] Report from the Johnston-Livingston Fund for Experimental Therapeutics in the Department of Experimental Therapeutics, Cornell University Medical College.

[2] American Jour. of Physiology, vol. xxxvi, no. 2, Jan. 1915, p. 113.

then evaporated to dryness and extracted with alcohol, and then only the alcohol soluble portion proved to contain the active material. This was then further subdivided into a lead precipitate and a lead filtrate; the latter liquid alone proved active in the same way and in the same proportion as the original first filtrate from which it was derived. Therefore, the first filtrate, which can be prepared with comparative ease and cheapness and was found to be sterilizable without altering its properties, has been employed for all subsequent experiments under the designation of the thyroid "residue." It signifies the portion of an aqueous extract of the thryoid which remains after the removal of the nucleoproteins, globulins and coagulable proteins. All the substances which can be separated from the thyroid by the means we have employed contain iodin of which the largest amount is found in the nucleoproteins.

As is well known, more iodin is present in the pig than in the sheep thyroid. When similar amounts of the pig and of the sheep "residues," calculated according to their nitrogen content, are injected intravenously, the pig thyroid residue produced a greater fall in blood pressure than the sheep "residue." When, however, the dosage, calculated according to the iodin content, is the same, the resultant fall in blood pressure is precisely the same. Hence the active vasodilating principle of the thyroid "residue" apparently contains iodin.

After studying the effects of the different materials which can be recovered from an aqueous extract of the thyroid, it has seemed advisable to compare the results with a similar series of experiments with the materials obtained in a corresponding manner from other organs. It was found that the nucleoproteins, globulins and coagulable proteins recovered from aqueous extracts of the thymus, pancreas and liver, like those from the thyroid, were entirely inert, while the filtrates or "residues," as they will hereafter be called, which remained after the removal of these bodies were uniformly active. Therefore, in the subsequent tests only the "residue" of organ extracts have been employed. Contrary to the experience of others who had used somewhat similar materials, we have not noted the estab-

lishment of any perceptible tolerance to these "residues" after their repeated injection.

All the "residues," or first filtrates, like that of the thyroid have shown a more or less marked depressor effect, except the "residue" prepared from the adrenal gland. This power of producing fall in blood pressure, possessed by watery extracts of so many organs has long been known and has generally been believed to be caused by a common cholin content. We have, therefore, prepared cholin by Hoppe-Seyler's method, and have found that an amount of cholin which is comparable in its nitrogen content to a thyroid "residue," for example, produces in the kymograph tracings no fall in blood pressure. Hence, the vasodilating principle is not cholin. Vincent[3] and others have demonstrated that cholin cannot be present in at least some of the extracts and, therefore, the depressor effect must be due to some other substance. This may be the same in every organ and be represented by what Popielski[4] designates as "vasodilatin;" or it may be a material peculiar to and characteristic of each organ. Popielski worked with the same organs as we have, but the material he isolated and used for his experiments differs much in its character and methods of preparation from our material. After a comparison of all the observations, however, it is more reasonable to believe in a common vasodilating principle than in a number of different principles having the same effect. It seems probable that a substance, some part or all of which has a vasodilating effect, exists in, or is produced by, all organs in amounts which vary in each. For in our experiments the "residue," or portion of the extract of each organ which is demonstrably active in the kymograph tracings, produces a fall in blood pressure which is more or less characteristic for the "residue" of the particular organ employed when standardized according to its nitrogen content and compared with a similar amount of thyroid "residue." The other differences which can be detected in the secondary rise after the primary fall in blood pressure (iv, pancreas residue, v, pituitary, viii, thymus), and

[3] Vincent: Int. Secretions and Ductless Glands, 1912, p. 31.

[4] Popielski: Chemical Abstracts, vol. 7, no. 4, Feb. 20, 1913, p. 613.

the alteration in the depth of respiration (i, thyroid, iii, liver, v, pituitary, vii, spleen), when compared with the characteristic fall in blood pressure, produced by similar amounts of each "residue," standardized according to its nitrogen content suggest that every organ is capable of producing some particular material which is active in the sense of having some specific effect. It is also possible that this material consists of a combination of some depressor substance common to all organs, with another body which constitutes a secondary and different active principle in every organ.

As it might be possible that the depressor agents in the "residues" were due to concentrated salts or to some form of protein which had escaped removal and so might be common to all "residues," we have tested the effects of intravenous injections of salts and proteins in varying dosage, but always with negative results. The preparations employed consist of sodium iodid, potassium iodid, calcium iodid, calcium citrate, sodium citrate, sodium chloride and Witte's peptone. In doses of 5 cc. to 10 cc. of a 2 per cent solution these substances do give a vasodilatation effect, but these amounts are so far in excess of any possible content in our "residues" that they cannot be taken into consideration. The "residue" from the adrenal gland has been found to contain epinephrin in a concentration of approximately 1 to 250, as compared to the 1:1000 solution of adrenalin chloride prepared by Parke, Davis & Co., and is the only "residue" which shows a pressor effect. That from the pituitary gland, standardized and compared with the others according to the nitrogen content, shows the most marked primary depressor effect which is followed later by a rise in blood pressure.

Both of these glands are peculiar in the different structure and different physiological action of their anatomically closely related parts, and its does not seem probable that either organ normally pours into the circulation two disassociated secretions. It is more reasonable to believe that the pressor principle, or the similar "pituitrin" and epinephrin exist during life in combination with some less easily demonstrable or secondary active principle which comes from the other half of each gland. If this is

true, the "residue" of these glands which correspond to all other "residues," should indicate it, and that of the pituitary which produces the greatest fall in blood pressure of all the "residues," and is followed by a secondary rise with marked deepening of respiration, is certainly suggestive. The adrenal "residue" is the only "residue" which produces a primary pressor effect, but its tracings differ materially from those of adrenalin.

The experiments have been made with the following extracts, standardized according to their protein content:

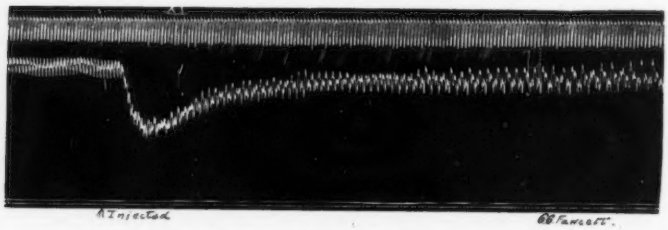
Residue	*Strength of solution in mgs. of protein in cc.*
Sheep thyroid.....	30.0 mgs. of nitrogen calculated as protein per cc. of solution
Pig thyroid.....	26.6 mgs. of nitrogen calculated as protein per cc. of solution
Parathyroid.....	16.0 mgs. of nitrogen calculated as protein per cc. of solution
Liver.....	170.0 mgs. of nitrogen calculated as protein per cc. of solution
Pancreas.....	157.5 mgs. of nitrogen calculated as protein per cc. of solution
Pituitary.....	16.0 mgs. of nitrogen calculated as protein per cc. of solution
Muscle.....	35.0 mgs. of nitrogen calculated as protein per cc. of solution
Spleen.....	20.0 mgs. of nitrogen calculated as protein per cc. of solution
Thymus.....	64.0 mgs. of nitrogen calculated as protein per cc. of solution
Adrenal.....	18.9 mgs. of nitrogen calculated as protein per cc. of solution
Cholin.....	6.0 mgs. of nitrogen calculated as protein per cc. of solution

When the "residues" are made under closely similar conditions, that is when the duration of the period in which the extract is made, the temperature at which it is conducted and the proportion of water to hashed gland and the degree of freshness of the organ, are all about the same for each gland extract, its content of nitrogen is quite characteristic of the organ from which that "residue" has been obtained. When a dose of "residue" from any organ is so calculated as to contain the same amount of nitrogen as the dose of thyroid "residue," the fall in blood pressure which it produces, when injected intravenously in a dog, is different for each of the organs tested. The resultant curves in the kymograph tracings, when the dose of the nitrogen content of each "residue" is kept the same, show distinct differences which seem peculiar to and characteristic of the organ from which the "residue" has been derived.

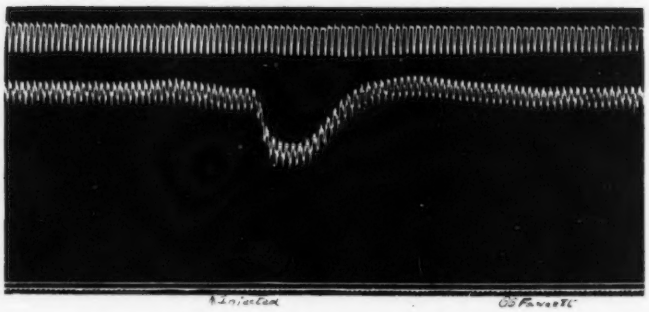
A summary of these results is given in the following table. The figures were all obtained from the same dog, as there are slight variations in different animals.

Residue	*Fall in blood pressure in mms. of Hg.*	*Mgm. of nitrogen calculated as protein*
Pituitary	69	26.6
Liver	58	26.6
Thyroid	52	26.6
Spleen	50	26.6
Thymus	48	26.6
Pancreas	40	26.6
Parathyroid	36	26.6
Muscle	32	26.6

The results of intravenous injections of these "residues" in quantities which contain the same amount of nitrogen are shown in the following tracings, numbered and labeled with the table to explain each. All the tracings are taken from one dog, with tracing XI as the check on the series.

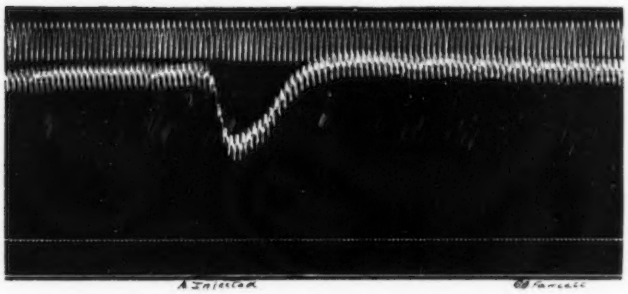


No. III. Liver residue. For dosage see table.

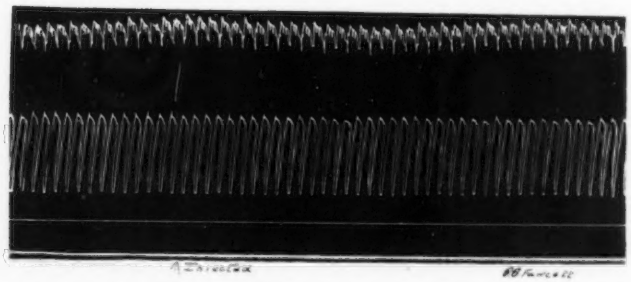


No. IV. Pancreas residue. For dosage see table.

No. V. Pituitary residue. For dosage see table.



No. VIII. Thymus residue. For dosage see table.



No. XVI. Cholin. For dosage see table.

TRACING NUMBER		I	II	III	IV	V	VI	VII	VIII	IX	X	XI NOTE I	XII NOTE II	XIII	XIV NOTE III	XV NOTE IV	XVI NOTE V
Extract used		Thyroid residue "pig"	Para-thyroid residue	Liver residue	Pancreas residue	Pituitary residue	Muscle residue	Spleen residue	Thymus residue	Thymus globulin	Thymus albumin	Thyroid residue "pig"	Thyroid residue "pig"	Cholin	Thyroid residue "sheep"	Thyroid residue "sheep"	Cholin
Strength of Solution in N. Calculated as Protein Mgs. per cc.		26.6	16.	170.	157.5	16.	35.	20.	64.								
Dosage	Minims	16.	26.6	2.5	2.7	26.6	12.12	21.2	6.6			26.6	17.5	16.	30.	30.	6.
	Mg's Protein	26.6	26.6	26.6	26.6	26.6	26.6	26.6	26.6	26.6	26.6	16.	5.	15.	3.	8.	32.
Effects on Blood Pressure	Fall in Mm's. Hg	52.	36.	58.	40	60.	32.	50.	48	0	0	26.6	5.1	5.1	5.1	15.	12.
	Rise in Mm's. Hg	0	0.	0	Secondary 6 mms.	Secondary 20 mms.	0	0	Secondary 6 mms.	0	0	50.	32.	0	22.	30.	0
	Time before Effect	6 sec.	4 sec.	4 sec.	6 sec.	6 sec.	7 sec.	6 sec.	4 sec.	0	0	0	0	0	Second 4 mms.	0	0
	Time of Action	2 min 5 sec.	3 min.	3 min. 20 sec.	1 min.	5 min.	35 sec.	1 min. 10 sec.	2 min.	0	0	3 sec. 2 min. 6 sec.	3 sec. 1 min. 30 sec.	0	4 sec. 44 sec.	4 sec. 44 sec.	0 0
Effects on Pulse Rate	Normal Rate per Minute	186	180	180	180	180	180	180	180	180	180	180	198	180	180	180	180
	Rate at Lowest Point of Bl'd. P.	183	176	198	180	168	180	180	176	0	0	180	194	180	180	180	184
	Rate during return of Bl'd. P. to Normal	186	180	198	180	174	180	180	174	0	0	180	185	180	184	180	184
	Rate after Bl'd. P. returns to normal	186	180	186	180	168	180	180	176	180	180	180	190	180	180	180	184
Effects on Respiration	Rate of Resp. at different Pts. of Bl'd. P. — Normal per Min	86	72	60	48	42	48	48	45	45	48	48	48	48	45	45	41
	Lowest point	86	72	60	44	42	48	48	45	45	48	48	48	46	48	45	41
	Returning to Normal	86	70	58	44	44	48	48	45	45	48	52	46	46	47	45	41
	Normal	86	66	58	46	46	48	48	45	45	48	48	48	48	47	43	41
	Amplitude of Resp. at different Pts. of Bl'd P. — Normal																
	Lowest point	Slight Increase	No Change	No Change	No Change	Normal Increase	No Change	—	No Change	No Change	No Change	Slight Increase	No Change	No Change	No Change	No Change	No Change
	Returning to normal					Increase		—									
	Normal					Decrease		Very Slight Increase									

Notes.

I. Thyroid residue was given here to check the condition of the dog's blood pressure.—The results are practically identical with Tracing No. I.

II. New preparation of thyroid "Residue" from pigs' thyroids. Iodin = 0.02 mgs. per cc.

III. Residue from sheeps' thyroids—Protein dosage same as Tracing No. XII.

IV. Iodin content of sheep-thyroid residue = 0.01143 mgs. per cc. Dosage of this made to correspond to iodin dosage of pig-thyroid residue of Tracing No. XII.
Iodin dosage of Tracing No. XII = .0062 mgs.
Iodin dosage of Tracing No. XV = .0062 mgs.

V. Dosage of Cholin = 12 mgs. which is more than twice the protein dosage of the thyroid residues of Tracings Nos. XII and XIV.

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CONCLUSIONS

(The term "residue" is used to designate that portion of an aqueous extract of an organ which remains after the removal of the nucleoproteins, globulins and coagulable proteins.)

1. The "residue" is the only part of an aqueous extract of the thyroid, thymus, pancreas, and liver which contains demonstrably active depressor agents, and it is, therefore, presumably true of all organs.

2. All the different organs from which we have prepared extracts, with the exception of the adrenal, contain a depressor substance within the "residue" portion, which in each case varies in its action directly as its nitrogen content (calculated in terms of protein).

3. The depressor effect of the thyroid "residue" is dependent upon a substance containing iodine.

4. When standardized according to its nitrogen content each "residue" produces characteristically different effects in the kymograph tracings.

5. This depressor agent cannot be cholin because a dosage of pure cholin which is comparable to that of a thyroid "residue" for example, produces no effect.

6. The active portion of these "residues" is apparently not the same as Popielski's vasodilatin, which is prepared in a totally different way and gives different reactions.

THE RATE OF OXIDATION OF ENZYMES AND THEIR CORRESPONDING PRO-ENZYMES

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This investigation was begun primarily to determine if there is any difference in the ease with which trypsin and trypsinogen, pepsin and pepsinogen can be oxidized.

TRYPSIN AND TRYPSINOGEN

Pancreatic juice was collected from dogs according to the ordinary method. One grain of morphia was injected hypodermically and subsequently the dog was etherized. A cannula was introduced into the pancreatic duct and one into the jugular vein of the animal. The mucosa was scraped from approximately 5 feet of the upper part of the intestines of two dogs for each experiment. This was hashed in a hashing machine and secretin prepared according to the method of Bayliss and Starling.[1] To the hashed mucosa were added 200 cc. of 0.4 per cent hydrochloric acid. The mixture was boiled and neutralized while boiling with 1 per cent sodium hydroxide. On filtering a clear filtrate was obtained. This was injected into the jugular vein of the etherized dog 5 cc. at a time at intervals of about five minutes. In this manner 100 cc. of perfectly clear pancreatic juice were obtained in the course of six hours.

The proteolytic activity of this juice was tested by adding a Mett's tube 1 mm. in diameter, 2 cm. in length, containing 10 per cent gelatine colored with Congo red. After standing at room temperature for forty-eight hours there was no indication of digestion.

[1] Starling: Principles of Human Physiology, 1912, 717.

A typical experiment may be described as follows: 100 cc. of pancreatic juice were collected according to the method given and divided into two portions of 50 cc. each. To one portion were added several crystals of thymol and 2 cc. of enterokinase. The enterokinase was prepared in the following way. A piece of intestine was slit open and thoroughly washed with tap water. The mucosa was gently scraped with the handle of a scalpel. This scraping was extracted with 25 cc. of 0.7 per cent sodium chloride. This solution was centrifugalized and filtered. In this way a fairly clear solution was obtained which possessed no proteolytic activity but was able to activate the trypsinogen solution.

The 50 cc. of juice to which the 2 cc. of enterokinase had been added was placed in the ice chest together with the 50 cc. to which no activator had been added. After standing in the ice chest for twenty-four hours the two solutions were tested for proteolytic activity by adding a Mett's tube of the type described above to 5 cc. of each of the solutions. It was found that the activated juice digested 5.2 mm. of gelatine in forty-eight hours at room temperature. The non-activated juice digested none of the gelatine.

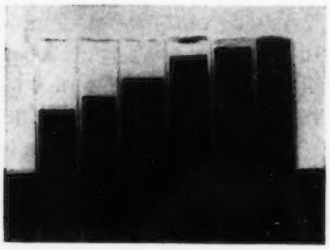
The object of the experiments to be described was to determine if the solution of trypsinogen were more easily oxidized than the solution of trypsin. The 50 cc. of pancreatic juice to which 2 cc. of enterokinase had been added was the trypsin solution and the 50 cc. to which no enterokinase had been added was the trypsinogen solution. Oxidation of the trypsinogen and trypsin was brought about by the oxygen liberated by the passage of the direct electric current through their solutions.

TRYPSIN

Five cubic centimeters of the activated pancreatic juice were introduced into an electrolytic cylinder,[2] a description of which had already been published in this JOURNAL. This cylinder was placed across the electrodes of a direct electric circuit in

[2] Burge: This Journal, 1913, xxxi, 328.

series with a potential reducer and a milliammeter. The cell was then fastened in a shaking machine and shaken at the rate of 250 double shakes per minute in order to prevent polarization while 25 milliamperes were passed for twenty-five minutes. The electrolyzed trypsin solution was then removed and 5 cc. of fresh trypsin solution introduced. The cylinder was replaced in the shaking machine and 25 milliamperes were passed for fifty minutes. At the end of this period this solution was removed and the cylinder recharged. Electrolyses were continued in this manner until trypsin solutions were obtained through which 37.5, 75, 112.5, 150 and 187.5 coulombs of electricity had passed. When the series was complete a Mett's tube was introduced into each of the electrolyzed trypsin solutions and into 5 cc. of the non-electrolyzed as a control. These solutions stood at room temperature for forty-eight hours when the Mett's tubes were removed and photographed. The reproduction of a photograph of the tubes of a typical experiment represents them approximately twice the actual size (fig. 1). The dark portion represents the amount of undigested gelatine; the light shows the empty tube from which the gelatine had been digested.



I II III IV V VI

Fig. 1. Photograph of Mett's tubes. The dark portion represents the undigested gelatine; the light, the extent of digestion.

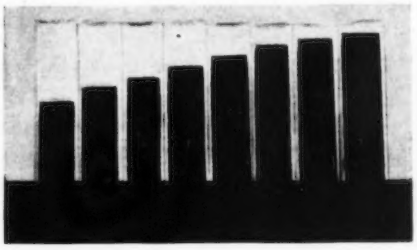
TABLE I

	TUBE	COULOMBS PASSED	MM. GEL. DIGESTED	DECREASE IN MM. GEL. DIGESTED	PERCENTAGE DECREASE IN DIGESTION
					per cent
Non-electrolyzed solution.....	I	0	5.0		
Electrolyzed solution.....	II	37.5	4.0	1.0	20.0
Electrolyzed solution.....	III	75.0	2.8	2.2	44.0
Electrolyzed solution.....	IV	112.5	1.8	3.2	64.0
Electrolyzed solution.....	V	150.0	0.5	4.5	90.0
Electrolyzed solution.....	VI	187.5	0.0	5.0	100.0

The extent of digestion in the same Mett's tubes, expressed in millimeters, is shown in the accompanying table (Table I). It may be seen that the amount of gelatine digested was decreased in proportion to the number of coulombs passed and that the passage of 187.5 Q. completely destroyed the activity of the trypsin.

TRYPSINOGEN

Trypsinogen solutions were electrolyzed in the manner described for trypsin. A series was obtained through which 37.5, 75, 112.5, 150 and 187.5 coulombs had passed. Three drops of enterokinase were added to each of the electrolyzed trypsinogen solutions and to 5 cc. of the non-electrolyzed for a control. These solutions were placed in the ice chest for six hours in order that the trypsinogen might be converted into trypsin. At the end of this time they were removed from the ice chest to room temperature and a Mett's tube introduced into each. After standing at room temperature for forty-eight hours the Mett's tubes were removed and photographed. The accompanying photograph shows the tubes of a typical experiment (fig. 2). Table II gives data regarding this same set of tubes. The extent of digestion in the different tubes is expressed in millimeters and the percentage decrease in digestive strength is given for the number of coulombs passed through the respective solutions.



I II III IV V VI VII VIII

Fig. 2. Photograph of Mett's tubes magnified twice. The dark portion represents the undigested gelatine; the light, the extent of digestion.

The conclusion to be drawn from these experiments is that trypsin is more easily destroyed by the passage of the direct electric current than trypsinogen and since the destruction of both of these substances is due to oxidation the further con-

clusion is justified that trypsin is more easily oxidized than trypsinogen.

TABLE II

	TUBE	COULOMBS PASSED	MM. GEL. DIGESTED	DECREASE IN MM. GEL. DIGESTED	PERCENTAGE DECREASE IN DIGESTION
					per cent
Non-electrolyzed solution	I	0	5.8		
Electrolyzed solution	II	37.5	4.5	1.3	22.4
Electrolyzed solution	III	75.0	3.8	2.0	34.4
Electrolyzed solution	IV	112.5	3.0	2.8	48.2
Electrolyzed solution	V	150.0	2.3	3.5	60.3
Electrolyzed solution	VI	187.5	1.5	4.3	74.1
Electrolyzed solution	VII	225.0	1.0	4.8	82.7
Electrolyzed solution	VIII	262.5	0.6	5.2	89.6

PEPSIN AND PEPSINOGEN

Experiments similar to those carried out using trypsin and trypsinogen were performed using pepsin and pepsinogen.

A typical experiment may be described as follows: Immediately after death the stomachs of two dogs were removed, slit open and washed thoroughly with tap water. These were immersed in a 1 per cent sodium bicarbonate solution for one minute in order to remove any pepsin still adhering and at the same time to harm the pepsinogen as little as possible. Langley and Edkins[3] have shown that pepsin is more easily destroyed by sodium bicarbonate than is pepsinogen. The stomachs were again washed with tap water and rinsed with distilled water. The mucosa was torn off, hashed in a hashing machine and ground up with sand in a mortar. Approximately 100 cc. of 0.7 per cent solution of sodium chloride were added to the hashed mucosa. This mixture was placed in the ice chest for twenty-four hours. At the end of this time it was placed in a press and the liquid portion removed. This liquid was centrifugalized and subsequently filtered through several thicknesses of fine grained filter paper by means of a vacuum pump. The solution thus obtained was moderately clear. This was

[3] Langley and Edkins: Journal of Physiology, 1886, vii, 371.

divided into two portions of 50 cc. each. To one portion was added 0.5 cc. of a 37 per cent solution of hydrochloric acid while the material was being shaken vigorously. This acid solution and the remaining 50 cc. were placed in the ice chest. After six hours both the activated and the non-activated extract of the gastric mucosa were placed in collodion diffusion tubes and dialyzed against running tap water for twenty-four hours. Each diffusion shell was then suspended in a vessel containing 10 liters of distilled water and dialyzed for twenty-four hours. At the end of this period both tubes were placed in one vessel containing 10 liters of distilled water and dialyzed for another period of twenty-four hours. The result of this treatment was that the conductivity of the two solutions was practically the same.

PEPSIN

The extract of the gastric mucosa to which hydrochloric acid had been added and which was subsequently dialyzed was the pepsin solution used. This solution was electrolyzed in the same manner as the trypsin and trypsinogen solution. Thus

TABLE III

	TUBE	COULOMBS PASSED	MM. GEL. DIGESTED	DECREASE IN MM. GEL. DIGESTED	PERCENTAGE DECREASE IN DIGESTION
					per cent
Non-electrolyzed solution.....	I	0	10.0		
Electrolyzed solution.....	II	36	4.0	6.0	60.0
Electrolyzed solution.....	III	72	1.0	9.0	90.0
Electrolyzed solution.....	IV	108	0.0	10.0	100.0

pepsin solutions were obtained through which 36, 72, and 108 coulombs of electricity had been passed. It may be recalled that these solutions had been made practically free of hydrochloric acid by means of dialysis, hence it was necessary to make the electrolyzed pepsin solutions acid before digestion could be begun. For this purpose 1 cc. of 1 per cent hydrochloric acid was added to 3 cc. of each of the electrolyzed pepsin solutions and to 3 cc. of the non-electrolyzed as a control. A Mett's tube was

introduced into each of the 0.25 per cent hydrochloric acid-pepsin solutions. These solutions were permitted to stand at room temperature for forty-eight hours. The number of millimeters of gelatine digested in that period in the Mett's tubes may be seen in the accompanying table (Table III).

It may be seen from the table that 10 mm. of gelatine were digested by the non-electrolyzed pepsin solution in forty-eight hours, and that no digestion had taken place at this time in the tube in the solution through which 108 coulombs of electricity had passed.

PEPSINOGEN

The extract of the gastric mucosa freed from hydrochloric acid and pepsin by means of 1 per cent sodium bicarbonate and subsequently dialyzed was the pepsinogen solution used. Electrolyses were carried out until solutions of pepsinogen were

TABLE IV

	TUBE	COULOMBS PASSED	MM. GEL. DIGESTED	DECREASE IN MM. GEL. DIGESTION	PERCENTAGE DECREASE IN DIGESTION
					per cent
Non-electrolyzed solution.....	I	0	7.0		
Electrolyzed solution.....	II	36	6.5	0.5	7.1
Electrolyzed solution.....	III	72	6.0	1.0	14.2
Electrolyzed solution.....	IV	108	5.0	2.0	28.5

obtained through which 36, 72 and 108 coulombs of electricity had passed. These pepsinogen solutions were then activated by the addition of 1 per cent hydrochloric acid. One cc. of the acid was added to 3 cc. of each of the electrolyzed pepsinogen solutions and to 3 cc. of the non-electrolyzed pepsinogen solution to serve as a control. These solutions were placed in the ice chest for six hours. During this time the pepsinogen was converted into pepsin. A Mett's tube was then introduced into each of the activated solutions and digestion allowed to proceed at room temperature. After forty-eight hours the tubes were removed and the extent of digestion measured. Table IV gives the measurements for a typical experiment.

It may be seen that 7.0 mm. of gelatine were digested in the non-electrolyzed control solution in forty-eight hours and that in the solution through which 108 coulombs had passed digestion had taken place to the extent of 5.0 mm.

It will be seen from these experiments that pepsin is more easily destroyed by the passage of the direct electric current than is pepsinogen and since the destruction of both of these substances by the passage of the current is due to oxidation we may conclude that pepsin is more easily oxidized than pepsinogen.

DISCUSSION

Lillie[4] and others have shown that the mucosa of the stomach and of the intestine possesses intense oxidative properties. We[5] have been able to show that all the ordinary digestive enzymes are easily oxidized by nascent oxygen. On the basis of these two facts we have advanced the hypothesis that the mucosa of the digestive tract maintains its integrity during life by rendering inactive the layer of enzyme solution immediately in contact with it. This assumes that there are two opposing activities at work, viz., the active enzyme within the lumen of the digestive tract attempting as it were to digest the cells of the mucosa while the oxidative processes of these cells are rendering the enzyme inactive and hence protecting the mucosa from digestion.

It has been shown that in diseases of the circulatory and respiratory systems,[6] in acute yellow atrophy of the liver and in chloroform and phosphorus poisoning the tendency of the tissues to undergo auto-digestion is greatly increased. It has also been shown that under these abnormal conditions the oxidative processes of the tissues are decreased.[7] The observation has frequently been made that if the blood supply be cut off from a circumscribed area of the stomach wall the pepsin of the gastric

[4] Lillie: This Journal, 1902, vii, 413.

[5] Burge: Ibid., 1914, 141, 146.

[6] Schlesinger: Hofmeiter's Beitrage, 1904, iv, 87.

[7] Welsch: Archives internationales de Pharmacodinamie et de Therapie, 1905, xiv, 211.

juice begins to digest this area. It is obvious that in such cases, the blood supply being cut off, the area is deprived of oxygen and hence pepsin is left free to act. Under such circumstances gastric ulcers may arise.

If one accepts the hypothesis that a balance normally exists between the digestive enzymes in the lumen of the alimentary tract and the oxidative processes of the cells lining this tract it is necessary to explain the fact that pepsinogen is not oxidized during the process of its secretion by the cells of the gastric mucosa and the fact that trypsinogen is not oxidized by the cells of the pancreas since the cells of both of these tissues possess oxidative properties. The explanation that suggests itself is that pepsinogen and trypsinogen are not so easily oxidized as the active pepsin and trypsin. The foregoing experiments prove that this is true.

CONCLUSIONS

1. Trypsin is more easily oxidized than trypsinogen.
2. Pepsin is more easily oxidized than pepsinogen.
3. The fact that trypsin and pepsin are relatively easy to oxidize makes it possible for the mucosa of the stomach and intestine to protect itself from digestion by means of its oxidative processes.
4. The fact that trypsinogen and pepsinogen are relatively difficult to oxidize prevents these substances from being oxidized in the cells of the pancreas and of the gastric mucosa during the process of secretion.

THE EFFECTS OF EPINEPHRIN INFUSION ON VASOMOTOR IRRITABILITY

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That adrenal deficiency results in vasomotor depression is well known. Two explanations of the phenomenon have been offered which assume that the essential factor is a reduction in quantity of circulating epinephrin. One—the "tonus" theory—supposes that the sympathetic system under normal conditions is kept in continuous activity by epinephrin stimulation. This theory is no longer tenable(1). Another possible explanation was proposed by Elliott in 1904 (2)—namely, that in the absence of epinephrin the ability of the "myoneural receptive substance" to transmit impulses from the sympathetic fibres to the muscle cells is lessened. In a previous research on partial adrenal deficiency (3) occasion was offered to test the effect of adrenalin infusion in two dogs showing vasomotor depression. The surprising fact was noted that while the animals were under the influence of small quantities of epinephrin the responses to vasomotor stimulation were actually depressed. The phenomenon seemed worthy of further investigation.

Accordingly we have studied the effects of adrenalin infusion upon vasomotor irritability in 42 other dogs. The animals were all anesthetized,—various methods being used: ether alone by open cone or by the ether-bottle, ether-urethane, and paraldehyde by stomach, supplemented when necessary by ether. The most satisfactory method, however, was decerebration. In some cases curare was injected by vein, particularly when sciatic stimulation was used, in which case it added materially to definiteness in results. In such instances artificial respiration of course had to be employed. For this purpose the Gesell

and Erlanger apparatus was used. Several of the earlier experiments were made upon dogs partially or completely deprived of their adrenal tissue. This procedure made no apparent difference in results, however, and in all the later cases was omitted. This finding incidentally is in harmony with our previous experience which indicates that aside from shock adrenal extirpation produces no immediate effects whatever. It supports our present belief that epinephrin in significant amount ordinarily is not present in the blood stream. The condition of the vasomotor apparatus before, during and after the adrenal infusion was tested by various methods of stimulation: faradization of the sciatic nerve and of the left splanchnic nerve; and intravenous injections of nicotin, adrenalin and pituitrin. The adrenalin for infusion was diluted with distilled water usually to a concentration of 1:100.000, but various other dilutions from 1:10,000,000 to 1:25,000 were also tried. The infusions were made from a burette connected with a hypodermic needle inserted in a vein—usually an external jugular. The rate of flow was regulated by raising and lowering the burette. Blood pressure was recorded from a femoral artery by the reservoir cannula method previously described(4).

While the results of the series of experiments as a whole are in harmony with our earlier findings, a great deal of variability was noted. In some instances the merest trace of epinephrin added to the blood stream was sufficient to cause a marked depression of vasomotor irritability; in other instances comparatively large quantities were required to cause any material decrease in reactions. In these later cases the augmented pressure due to the adrenalin might in itself, if they stood alone, be regarded as the sole cause of decreased reaction to further stimulation. A considerable number of animals were intermediate between the two extremes. For instance, a given dose of nicotin might cause a rise of 40 mm. of pressure. If now adrenalin was infused at a rate to raise the general pressure level 20 mm., a repetition of the dose of nicotin would cause a further rise of 20 mm., but no summation. When the adrenalin was discontinued the nicotin again produced its original effect, 40 mm.

rise. In other words the nicotin stimulation plus the stimulation due to the infused adrenalin was no greater than that of the nicotin alone. That there is a depressing factor involved in such results is shown by the fact that when a given dose of adrenalin and of nicotin were mixed and injected together a greater effect was produced than that due to either acting alone. It follows, therefore, that the effect of adrenalin infusion plus nicotin injection is a resultant of three factors: nicotin stimulation, adrenalin stimulation and adrenalin depression of irritability the depression developing more slowly than the stimulation. In a specific case, as a matter of fact, it was found to require

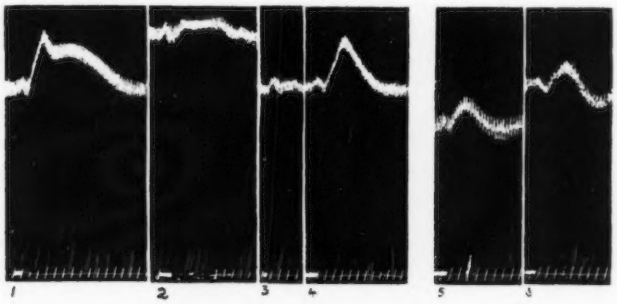

Fig. 1. Adrenalin infusion, nicotin stimulation. At 1, 2, 3, 4, 5 and 6, 2 cc. nicotine 1:4000, injected by femoral vein. 1, normal; 2, during adrenalin infusion; 3, during adrenalin infusion and amyl nitrite inhalation; 4, normal; 5 and 6, later experiment, same animal; 5, during amyl nitrite; 6, immediately after. Infusion adrenalin 1:100,000 by jugular vein, 2.5 cc. per minute. Blood pressure from femoral artery. Dog weight 5 kilos. Anesthesia, ether. Base line: 0 pressure, signal and time (5 secs.) Reduced to ½.

about three minutes for the depression to develop after the infusion began, although usually it occurred almost immediately.

The confusing condition in such cases is of course the rise of pressure that accompanies the epinephrin infusion. It is possible, however, by the use of amyl nitrite to counteract this pressor effect, leaving the depressor influence plainly in evidence. Figure 1 illustrates such a case. The animal was given 2 cc. of nicotin, 1:4000, by vein. The result is shown in graph 1. Then

adrenalin, 1: 100,000, was infused at the rate of 2.5 cc. per minute. After one and one-half minutes infusion a repetition of the nicotin produced almost no effect (graph 2). While the adrenalin was still flowing the animal was made to breathe amyl nitrite to bring the pressure down to the original level and the nicotin injection repeated. Again the nicotin produced little effect (graph 3). The interval between injections was three minutes. The amyl nitrite was discontinued and the pressure again rose to the height shown in graph 2. The adrenalin infusion was then discontinued and the pressure fell to the original level. One and three-fourths minutes after the adrenalin was stopped a repetition of the nicotin injection gave its original effect (graph 4). That the amyl nitrite was not itself the cause of the block in graph 3 is shown by graphs 5 and 6 obtained later from the same dog. They show the effects of the same dose of nicotin during and after amyl nitrite inhalation. There is no appreciable difference in the two cases.

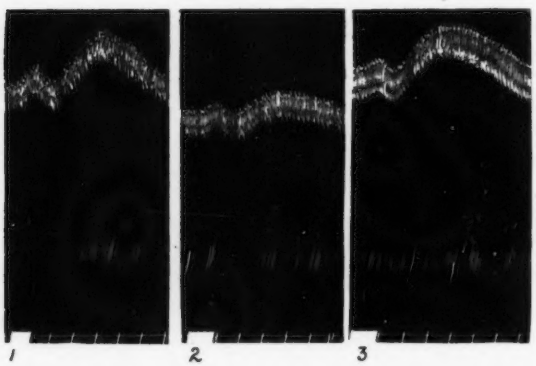

Fig. 2. Adrenalin infusion, nicotin stimulation. Nicotin, 1:4000, 1 cc. by femoral vein. 1, before; 2, during, and 3, after adrenalin infusion, 1: 100,000, 2.6 cc. per minute by jugular vein. Blood pressure from femoral artery. Dog weight 9 kilos. Anesthesia, ether. Base line: 0 pressure, signal and time (5 secs.). Reduced to ½.

Figure 2 illustrates the convincing results obtained in an animal that was unusually sensitive to adrenalin. Graph 1 shows the effect of 2 cc. of nicotin, 1:4000. Adrenalin infusion 1:200,000 at the rate of 2.6 cc. per minute caused a slight lowering of the general blood pressure level. Two and one-half minutes after the infusion began the nicotin stimulation was repeated and

caused only a slight rise (graph 2). When the adrenalin was discontinued the pressure returned to its previous level and again (graph 3) nicotin caused the same rise as before.

That the depression of irritability is a specific effect of the adrenalin was shown in another way. In an animal that gave a clean-cut decrease in the nicotin reaction during adrenalin infusion the conditions were reversed. Nicotin was used as the infusion agent, a rate being selected that closely simulated the effect of the adrenalin infusion. Adrenalin was injected as the stimulating agent, before, during and after the infusion. No depression of the reaction occurred.

Having established the fact that epinephrin produces a depression of vasomotor irritability an attempt was made to determine the locus of the block. Different components of the vasomotor apparatus were stimulated by appropriate means and the effects of adrenalin infusion noted.

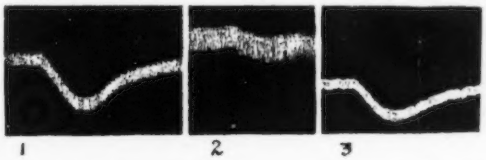

Fig. 3. Adrenalin infusion, sciatic nerve faradized. Stimulation at 1, before; 2, during, and 3, after infusion with adrenalin, 1:50000 2.3 cc. per minute, by jugular vein. Interval between stimulations, 2 minutes. Blood pressure from femoral artery. Dog weight, 7 kilos. Anesthesia, ether. Initial blood pressure 87 mm. No reduction.

As an example of the afferent nervous components the sciatic was selected. This was exposed through an incision in the posterior aspect of the leg at the mid-femoral region. A Harvard shielded electrode was applied and packed off with dry cotton. A tight ligature was tied around the nerve distal to the electrode and the incision closed. In some cases curare was administered and in others not; the results were qualitatively the same in both cases but quantitatively greater in the curarized dogs. The nerve was stimulated by induced currents of various degrees of strength to produce in some instances a pressor and in others a depressor effect. The proper position of the secondary coil having been determined in each instance the effect of stimula-

tion before, during and after adrenalin infusion was determined. When pressor stimulations were employed results closely similar to those obtained with nicotin were secured. These therefore need not be further described. Figure 3 show the results of an experiment in which depression resulted from the stimulation. The rate of adrenalin infusion (1:50,000) was 2.3 cc. per minute. This caused a slight rise of the pressure level. The intervals between stimulations were two minutes. The graphs show the effects of stimulation (1) before, (2) during and (3) after infusion. The sciatic nerve experiments as a whole showed that both the pressor and depressor mechanisms are blocked and indicate that the results obtained in the nicotin injections are

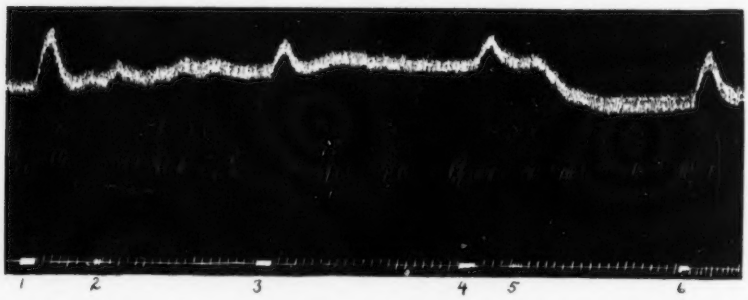

Fig. 4. Adrenalin infusion, splanchnic nerves faradized. At 1, 3, 5 and 6 stimulation. At 2, infusion begun. At 5, infusion discontinued. Adrenalin 1:100,000, 5.7 cc. per minute. Blood pressure from femoral artery. Dog weight, 11 kilos. Anesthesia, ether. Base line: 0 pressure, signal and time (5 secs.). Reduced to ½.

not to be ascribed to any sort of chemical interaction among the drugs employed.

Figure 4 shows the results obtained in an experiment in which stimulus was applied to the peripheral (post-ganglionic) nervous elements. In this case the left splanchnic trunks were isolated in the region of the adrenal gland. The adrenal was tied off. A shielded electrode was adjusted to the nerves which were tightly ligated centrally. The electrode was surrounded with dry cotton to absorb any fluid that might accumulate and

the incision closed. At 1 a faradic current was sent through the electrode. At 2 infusion with adrenalin 1:100,000 was begun. At 3 and 4 the stimulation was repeated. At 5 the adrenalin was discontinued and at 6 the stimulus was repeated. The same strength of current of course was used throughout. The reaction was depressed about 50 per cent. Other experiments gave similar results.

According to Pilcher and Sollman's late work (6) nicotin stimulates the vasomotor centre but according to Langley and Dickinson (7) the effect is chiefly on the ganglia of the sympathetic system. The foregoing evidence as a whole indicates that adrenalin depression is shown when the stimulus is applied to any part of the vasomotor system proper. This fact indicates that the block is in part at least peripheral, either in the myoneural junctions or the smooth muscle itself. The fact, however, that both the pressor and depressor mechanisms are affected is most easily explained on an assumption that the depression is partly central (compare figs. 2 and 3).

Our attempts to settle the point by direct experiment were not entirely successful. In several cases both nicotin and adrenalin injections were made during adrenalin infusion. Usually the nicotin reaction was more depressed than was the adrenalin reaction, but depression was shown in both cases. Such findings are hard to interpret because the adrenalin stimulation was effected through the myoneural junction, a structure that was already undergoing excitation by the infused adrenalin, whereas, in case of the nicotin stimulation the myoneural junction was merely transmitting the impulses. To what extent the transmission involves also excitation is undetermined. In a single instance we have noted in the course of another research not yet published that myoneural transmission may remain unimpaired when myoneural excitability is markedly decreased. To state the case specifically, it was noted that after injection with a thyroid preparation an animal lost the greater part of its irritability to adrenalin while the reactions to pituitrin and to nicotin were unaffected.

The reaction to pituitrin which stimulates the smooth muscle

only was also investigated. Several difficulties presented themselves in such experiments. Repeated doses of pituitrin have to be small or they soon cease to be effective, and with a small initial stimulus the effects of adrenalin infusion were difficult to demonstrate. Also, the doses can not be repeated at short intervals as can adrenalin and nicotin and the condition of the experimental animal is likely to change from one injection to the next. Comparative studies of the relative effects of the infusion upon the pituitrin and the other reactions are not conclusive because pituitrin itself, according to Keponow (8), alters the excitability of the vasomotor mechanism. So far as could be judged, however, in the face of these difficulties, the vasomotor reaction to pituitrin is also somewhat depressed by adrenalin infusion. The depression therefore is probably both central and peripheral.

Our first experiments were made to determine whether adrenalin has any ability to *facilitate* sympathetic functioning. Considering the fact that various instances of reversal of effect have been reported with change of dosage it was not *a priori* unlikely that very high dilutions might augment irritability even in those animals that showed depression with greater quantities. In the first experiments of this series the matter was investigated. The infusions were begun with very dilute solutions, e.g., 1:20,000,000, and the strength gradually increased by stages to an effective concentration. In one or two instances there was noted slight augmentation in the reactions to stimulation but this was within the limits of experimental error. Ordinarily the first effect to be seen was depression. It appears therefore that epinephrin has little or no ability to increase vasomotor irritability or the transmission of vasomotor impulses.

The vasomotor depression which was noted developed quickly after the epinephrin infusion began, and was as quickly recovered from when the infusion was stopped. In one instance a lag of two or three minutes was noted but generally the lag was not more than half a minute.

That reflex discharge of epinephrin from the animal's own glands is not a significant complicating factor in our results is

indicated by three facts: the results in several instances in which the animal's glands were removed were similar to those in normal animals; the latent period after stimulation was shorter than that necessary for the stimulus to produce its effects *via* the adrenals; and usually, the secondary waves in the pressure curve that are characteristic of adrenal discharge did not occur. Moreover, in most cases a secondary discharge of epinephrin would have made no difference in the significance of the results.

Our results are possibly merely a phase of the depressor influence of epinephrin recently studied by Hoskins and McClure (9) in dogs and by Cannon and Lyman (10) in cats. These investigators have corroborated previous reports that under suitable conditions of dosage and rate of injection adrenalin characteristically produces a fall of blood pressure. Cannon and Lyman are inclined to attribute the depression to an influence on the vascular muscle cells. In a preceding paragraph we called attention to evidence that the depression is partly central, but our results also indicate a peripheral effect.

As to the significance of our findings we have little comment to offer. They are quite at variance with what we had first expected. They are scarcely reconcilable with any of the more popular theories as to the functions of the chromaffin system. They seem to indicate that the presence of small quantities of epinephrin in the circulating blood is not only of no use to the individual but in many cases at least is actually detrimental. If this be true it is quite probable that under ordinary conditions epinephrin does not exist in the blood at all. Under conditions of special stress, however, when the adrenal glands are discharging relatively large amounts of their secretion, the depressing influence as compared with the stimulating effect would be slight and probably neglible.

SUMMARY AND CONCLUSIONS

1. In 44 anesthetized dogs the effects of adrenalin infusion upon the irritability of the vasomotor mechanism was investigated.

2. The vasomotor mechanism was stimulated by faradization of the sciatic and splanchnic nerves, and by injections of nicotin, adrenalin and pituitrin, before, during and after intravenous infusion with adrenalin.

3. No concentration of adrenalin gave satisfactory evidence of augmenting vasomotor irritability, or facilitating the transmission of vasomotor impulses.

4. In most cases the infusion lessened the vasomotor irritability—sometimes to a marked degree.

5. The animal's own adrenal glands played no significant part in the results.

6. The irritability of both the pressor and depressor mechanisms was decreased.

7. The depression was probably both central and peripheral.

8. Circulating epinephrin is probably not a factor in the ordinary functioning of the animal economy.

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(2) Elliott: Journal of Physiology, 1904, xxxi, p. xx.
(3) Hoskins: This Journal, 1915, xxxvi, 423.
(4) Hoskins and Wheelon: Ibid., 1914, xxxiv, 81; Hoskins, ibid., 1915, xxxvi, 424.
(5) Gesell and Erlanger: Ibid., 1914, xxxiii, p. xxxiii.
(6) Pilcher and Sollmann: Journal of Pharmacology and Experimental Therapeutics, 1915, vi, 369.
(7) Langley and Dickinson: Journal of Physiology, 1890, xi, 297.
(8) Kepinow: Archiv für experimentelle Pathologie und Pharmakologie, lxvii, 247.
(9) Hoskins and McClure: Archives of Internal Medicine, 1912, x, 343.
(10) Cannon and Lyman: This Journal, 1913, xxxi, 376.

THE DISTRIBUTION OF GASTRIN IN THE BODY

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A. INTRODUCTION AND LITERATURE

Since the almost simultaneous reports in 1906 by Edkins and Gross that a gastric secretin probably is a factor in stimulating gastric juice secretion further work on this phase of secretagogue action had not been reported until recently. This later work was not known to us and most of it had not been published at the time these investigations were begun in the summer of 1912.

Edkins[1] demonstrated that 0.4 per cent hydrochloric acid extracts of the cardiac or pyloric mucous membrane from hogs' stomachs when intravenously administered to an anesthetized cat cause a secretion of gastric juice, but that similar extracts from the fundus portion are inactive. He further stated that extracts prepared by using cold water, peptone, glucose, or glycerol solutions as solvents also contain variable amounts of this same secretagogue which he called gastrin. Gross[2] in the Pawlow laboratory also suggested that a gastric hormone may be involved in gastric secretion in that he found beef extract to cause a flow of gastric juice only when introduced into the pyloric part of the stomach, and not when introduced into the fundic part. Popielski[3] in a series of individual and joint papers repeatedly

[1] J. S. Edkins: Jour. of Physiol, 1906, xxxiv, 133.

[2] Walter Gross: Arch. f. Verdauungskrankheiten, 1906, xii, 507.

[3] L. Popielski: Pflüg. Arch., 1909, cxxvi, 483; ibid., 1909, cxviii, 191; L. Popielski and K. Panek; ibid., 1909, cxxviii, 222; L. Popielski: Centrbl. f. Physiologie, 1910, xxiv, 635; ibid., 1910, xxiv, 1102; Centrbl. f. Biochem. u. Biophys. 1910–11, xi, 724; Pflüg. Arch., 1912, cxliv, 135; Pflüg. Arch., 1913, cl, 1.

calls attention to the general distribution in tissues and in Witte's peptone of a substance or of substances which cause vasodilation when introduced intravenously; also that coincident with the fall in blood pressure we have a decreased coagulability of the blood and a more active secretion in the digestive glands in general. He considers the secretagogue action as due not to a specific effect on the cells, but to the more fluid (more "filterable") condition of the blood as well as to the vasodilatation. This substance or these substances so generally distributed he called "vasodilatin." He reported finding vasodilatin in brain, gastric and intestinal mucosa, pancreas, defibrinated blood, extracts from erythrocytes and in Witte's peptone. He found the hetero-, deutero- and proto-albumose fractions free from the substance and considers the activity due to a hydrolytic product from the proteoses or peptones. He does not report detailed comparative quantitative studies on the effects of these extracts on gastric secretion. Thus in his comparative physiological study on the effects of these various extracts he reports three experiments showing that 5 to 6 cc. of 5 per cent extracts in 0.4 per cent hydrochloric acid from the mucous membrane of the large intestine and rectum when injected intravenously (subcutaneously in one experiment) into dogs with oesophageal and gastric fistulas, caused an increased flow of fluid from the stomach as high as 18 cc. in one-fourth of an hour, but in no case was the acidity or peptic activity reported as measured before or after the injection. He does not report the effect of a similar extract from the gastric mucosa, but repeatedly states that large doses of peptone, intestinal or gastric mucosa extracts not only do not stimulate the flow of gastric juice, but may actually inhibit it. He criticizes Edkin's negative findings with fundus mucosa extract as possibly due to an overdose of "vasodilatin." In his most recent paper Popielski again calls our attention to the same general action of tissue extracts and also to the fact that increased submaxillary secretion is always accompanied by a decreased coagulability of the blood flowing from the gland, no matter whether the gland is stimulated to secretion by acid in the mouth, by pilocarpine or by stimulation

of the chorda tympani. In this connection it is interesting to note Popielski's statement that although imid-azol ethylamine (Modrakowski[4]), large doses of atropin, or stimulation of the depressor nerve cause a fall in blood pressure, still we do not observe a decreased coagulability of the blood or an increased secretion by the digestive glands. On the other hand, that when decreased coagulability of the blood accompanies a fall in blood pressure as in the action of vasodilatin, in blood transfusion or in anaphylaxis we do have an increased secretion by these glands. Popielski has not separated vasodilatin in pure form, but considers the substance to be different from cholin. From the researches of Popielski and his coworkers we are not able definitely to state what the factors are which are involved in secretagogue action; we are not able to state definitely that vasodilatin is the secretagogue, nor are we able to say whether the action is due to one substance which is present in different concentrations in various tissues, or whether to different substances with quantitatively different specificities, but not absolutely specific in any case.

A report by Mironescu[5] on intestinal secretion stimulation is of interest in this connection. A qualitative study led him to conclude that acid extracts from the rectum, large and small intestines, oesophagus, fundus, pylorus, duodenum, salivary gland, liver and suprarenals cause intestinal secretion in a Thiry-Vella fistula dog. Similar experiments from brain, pancreas, muscle or heart and peptone solutions he found inactive. The actual rate of flow and the character of the juice obtained were not considered in these studies, a more rapid flow for a short period of time was taken as a positive response. It is hardly necessary to state that studies on stimulation of intestinal juice secretion involve many complications and that a positive finding may easily be due to indirect secondary stimulations. Eisenhardt[6] has in part confirmed Edkins' findings. He employed

[4] G. Modrakowski: Arch. f. exper. Path u. Pharm., 1912, lxix, 67.

[5] Mironescu: Inter. Beitr. z. Path. u. Therap. Ernährungstörungen, 1910, i, 194.

[6] W. Eisenhardt: Inter. Beitr. z. Path. u. Therap. d. Ernährungsstörungen, 1910, i, 358; ibid, 1910, ii, 203.

dogs with Heidenhain-Bickel accessory stomachs and found the injection of dog gastric juice to cause a distinct but short rise in gastric secretion, providing the juice injected had been collected from a gastric fistula. Juice collected from the accessory fundus stomach possessed no secretagogue action. So also juice collected from the entire stomach when digested with casein or lactalbumin for one to five hours no longer possessed the hormone action, but if digested longer than five hours it was again found active. The fundus juice, however, could not be made active by such treatment, nor by more prolonged incubation with the protein. He reports negative results with the subcutaneous injection of predigested proteins (by acids), sodium oleate, lactose, glucose, saccharose, sodium chloride, commercial pepsin and gliadin digested thereby. On the other hand whey from milk and the dialysate from the whey reacted positively; a water extract from horse flesh reacted doubtfully, but a similar extract from roasted barley or wheat was followed by a good response; a press juice from spinach caused a very marked secretion. The author does not give quantitative data as to actual yield or nature of juice, nor as to the concentrations or amounts injected. R. Ehrmann[7] finds subcutaneously injected extracts from pyloric, fundic and duodenal mucosa in $\frac{N}{10}$ hydrochloric acid to cause a flow of gastric juice in Pawlow stomach dogs. He could not definitely distinguish differences as to degrees of activities due to marked variations and irregularities in the results. The maximum effect was noted in fifteen to thirty minutes after the injection. He reports negative results with Witte peptone, sodium nucleate, sarcosin, creatinine, alanin and glycocoll, Liebig's meat extract in doses of 0.5 to 1.0 gram were without effect, but a dose of 6.2 gram caused gastric secretion.

Otto Emsmann[8] from studies on dogs with Heidenhain pouches considered the gastric secretin to be present in the acid extracts obtained from the pylorus, duodenum, jejunum-ileum, liver and

[7] R. Ehrmann: Inter. Beitr. z. Path. u. Therap. d Ernährungsstörungen, 1911–1912, iii, 382.

[8] Otto Emsmann: Inter. Beitr. z. Path. u. Therap. d. Ernährungsstörungen, 1912, iii, 117.

pancreas, but mainly in the pylorus, duodenum and liver, the liver extract being very nearly as active as the pylorus extract. Emsmann's methods as reported are not quantitative enough to definitely establish this. The total secretion after an injection and the character of the secretion were not determined.

Z. Tomaszewski[9] in a preliminary report finds extracts from pyloric and fundus mucosa to cause a flow of gastric juice when administered to vagotomized dogs with gastric and duodenal fistulas. He studied quantitatively the quantity and acidity of the juice obtained; the peptic activity was not reported. B. E. Maydell[10] found extracts from the pyloric mucous membrane when subcutaneously administered to chronic gastric fistula dogs, to cause a flow of gastric juice; the amounts varied from $12\frac{1}{2}$ to 387 cc. per hour, the period of collection being one to one and one-half hours after injection. The acidity of the juice thus obtained was in general of the same order as pyschic juice from oesophageal fistula dogs, but the peptic activity (by Mett method) of the latter juice always was over twice that of the gastrin juice. Maydell reports negative findings with physiological salt solution, neutralized gastric juice, fundus extract and pancreatic secretin. It is hardly necessary to state that the purest preparations of pancreatic secretin thus far reported on have not been found to stimulate gastric secretion and that the pancreatic secretion brought about is of an entirely different character from that brought about by cholin or the so-called vasodilatin.[11, 12] To be sure a secretin solution showing *no* blood pressure lowering action has as yet not been obtained.

B. EXPERIMENTAL METHODS

(*a*) *Preparations.* In the studies cited above no attempts were made to carry out comparative physiological studies with other than the very crudest extracts. It was our purpose to

[9] Z. Tomaszewski: Zentbl. f. Physiol., 1913, xxvii.
[10] B. E. Maydell: Pflüg. Arch., 1913, cl, 390.
[11] Bayliss and Starling: Jour. of Physiol., 1902, xxviii, 325.
[12] Dixon and Hamill: Jour. of Physiol., 1909, xxxviii, 314.

first find a method which will yield a fairly active and stable preparation and still remove foreign substances, including vasodilatins as much as possible. Having established this we then sought to determine fairly quantitatively the specificity or distribution of the secretagogue in widely different tissues, and also to quantitatively follow the nature and quantity of the secretion obtained.

The method adopted after a number of preliminary experiments is based mainly on observations which have been made on the preparation of extractives in general and particularly on the preparation of pancreatic secretin claimed to be fairly free from vasodilatin. Briefly, the well washed fresh material was hashed and mixed with five times its weight of 0.4 per cent hydrochloric acid, then heated to 90° C. on the steam bath, set aside to cool to room temperature, the next day again heated to 90° C. and after again cooling and standing for twenty-four hours, filtered. The noted volume of recovered filtrate was then concentrated under diminished pressure to $\frac{1}{18}$–$\frac{1}{10}$ its original volume. Next we added six volumes redistilled 95 per cent alcohol, allowed to stand one to three days, filtered and the filtrate evaporated to dryness under diminished pressure. After dehydrating the residue by evaporating three times with 25 to 75 cc. portions of absolute alcohol, the material was extracted three or four times with boiling absolute alcohol. The residue insoluble in absolute alcohol was dissolved in water and again evaporated to dryness under diminished pressure. This treatment with water was repeated two or three times to remove the alcohol. Finally the residue was dissolved in water, filtered and diluted so that 1 cc. represented approximately 4 to 5 grams fresh tissue. This solution was then sterilized three or four times in sealed tubes on successive days. Hog tissues were used throughout.

(*b*) *Animals, methods of injection, dosage.* The advantages of testing the activity of gastrin preparations without the complicating factors of an anaesthetic are so obvious that they need not be dwelt upon. For these reasons it was decided to use dogs having Pawlow accessory stomachs and gastric fistulas.

This makes it possible to run many preparations on the same test stomach, thus eliminating the variations of the different individuals. The gastric fistula was prepared by establishing an opening through the left rectus muscle into the anterior wall of the fundus in the line of the anastomoses of the arterioles from the two curvatures of the stomach. Such animals maintain themselves in perfect health after the development of a functional sphincter of scar tissue about the fistulous opening. The Pawlow accessory stomachs were prepared and cared for in a manner which has been previously described by one of the authors.[13]

It was important, of course, to have the stomachs empty and secretorily quiescent before an experiment was to be run. Boldyreff[14] has shown that during certain fasting intervals there is a marked secretion of juice. While we have made no attempt to study this phase of the subject, our experience has shown that a fasting stomach frequently displays appreciable activity. Thinking that such activity might be associated with residual food particles in the mucosal folds, we first washed out the stomachs, one or two hours before the experimental periods. This procedure, instead of stopping a low grade secretion, apparently threw the mechanism into a more active state, so that secretion might last one, two, or three hours longer. The plan, which gave the most satisfactory results and which was finally adopted as a routine measure, consisted of feeding the animals heavily thirty-six hours before the experiment, and eighteen hours later giving each a pint of milk.

Animal holders were found to be a necessity, for only in this way can one be certain of the quantities of the juice. The dogs can be made quite comfortable, since they sleep during a good portion of the experimental period. It is important that juice be collected over a control period of two hours before the injection is made, so as to determine whether the trend of the stomach is to a higher or lower secretory level.

[13] R. W. Keeton: This Journal, 1914, xxxiii, 25.

[14] W. Boldyreff: Ergebnisse der Physiologie, 1911, xi, 121.

Intravenous injections into the leg vein caused some respiratory distress, and proved to be rather transient in their action. The intramuscular administrations into the lumbar region on the other hand presented no such difficulties, so this method was adopted. It is needless to say that with any long series of experiments a rigid aseptic technique and a sharp needle must be employed, if the dogs' backs and dispositions are to be kept in a satisfactory shape. The dosage usually consisted of 1 cc. of the preparations, which was doubled in the cases of very large animals.

C. ANALYTICAL METHODS

The collections of the juice were made hourly. The acidities were determined by titration with $\frac{N}{10}$ NaOH, and the percentages were expressed in terms of HCl as "free acidity" and "total acidity." In estimating the former, dimethyl-amido-azo-benzene was used as an indicator, and phenolphthalein for the latter. The expression of the results in terms of HCl facilitated the calculations for the pepsin estimations.

In estimating pepsin the Mett's tubes were used, under the modifications advised by Cobb.[15] A total volume of 3 cc. consisting of 1.5 cc. of juice and an equal amount of a diluent, was employed. The diluent consisted of the necessary volumes of $\frac{N}{5}$ HCl and water to bring the final acidity of the mixture up to 0.3 per cent HCl. The free acidity of the juice was used as a basis for this dilution. The tubes were incubated for twenty hours, and the peptic activity obtained by adding the millimeters of albumin digested at either end. Following Schütz' law, the square of the digestion in millimeters was multiplied by the number of cubic centimeters of juice secreted in order to obtain the peptic units of a given sample. The peptic activity represents the square of millimeters of albumin digested.

[15] P. W. Cobb: This Journal, 1905, xiii, 448.

D. RESULTS

1. The nature of the secretory response

An examination of the appended protocol shows the essential characters of the response to gastrin stimulation.

Dog IV. Gastric fistula. Weight 25 kilos

10.30	dressed			
11	11.0 cc. mucus; free acidity 0.000,		Total 0.027	Peptic act. 0.0
11.01	Injected 2 cc. fundus gastrin			
11.15	11.0 cc.	Free acidity 0.018	Total 0.109	32.49
11.30	23.0 cc.	0.319	0.391	8.41
11.45	15.0 cc.	0.419	0.474	5.76
12	13.0 cc.	0.346	0.401	4.84
12.15	9.0 cc.	0.228	0.309	8.41
12.45	1.4 cc.	0.255	0.291	

It will be noted that the latent period of the secretion is ten to fifteen minutes. The maximum quantity is secreted in the second fifteen minute period, and the maximum acidity is reached in the third quarter. When collections were made hourly, the juice of the first collection was usually larger in quantity, but of slightly lower acidity, than that of the second hour. Very rarely do the stimulating effects last longer than two hours, usually one and a half. The rise in acidity follows closely the increase in quantity. Indeed these two factors can be safely relied upon as criteria of the state of the mechanism, a fact which falls in line with Pawlow's contention[16] that the acidity of a given sample of juice is a function of the rate of secretion. This principle has recently been shown to hold for the human stomach by Reyfuss and Hawk.[17]

The pepsin content of a given sample of juice is apt to be rather misleading at first glance. The pepsin concentration may either rise or fall, following the injection of gastrin, depending on the relative quiescence of the glands before the injection, and the amount of fluid secreted. As a rule the fasting secretion of Pawlow stomachs consists of a thick viscid mucous material

[16] J. P. Pawlow: The work of the digestive glands. London, 1902, p. 31.

[17] M. E. Reyfuss, Olaf Bergheim and P. B. Hawk: Jour. Am. Med. Assn., lxiii, no. 24, 2088.

containing little or no acid, but high in pepsin. If now this type of stomach is stimulated with gastrin, the pepsin concentration falls, while the total number of peptic units secreted rises, which after all is the important thing. The pepsin secreting mechanism coördinates itself less closely with the acid and quantity factors, than do these latter two with each other. At times the former cells appear never to become quite quiescent, and at other times they spring into activity from a stimulus that does not affect the quantity output. This is shown in the preceding protocol. In the first fifteen minutes period the peptic activity was 32.49, and in the second 8.41. In other words, the pepsin production was going on full blast, before the acid and quantity production. Two experiments on a cat with a Pawlow stomach showed the same facts. In the first case, before the injection there was a digestion of 3 mm. (peptic activity 9.0), and after the injection, with no change in quantity of juice secreted, there was a digestion of 8.4 mm. (peptic activity 70.56). In a gastric fistula stomach one is more liable to find the fasting secretion free from residual pepsin, hence a stimulation will show an increase in both the total peptic units and the peptic activity (concentration per cc.). The variability of the factors controlling the pepsin secretion, and the lack of refinement of methods for measuring it, do not warrant an extended discussion of its secretion at present. However, the subject can be rested with this statement, that in all cases an injection of gastrin will increase the total output of pepsin.

2. Comparison of gastric fistula and Pawlow stomachs

At the outset of the work, it soon became evident that the method of response to stimulation of Pawlow and gastric fistula stomachs was different in many respects, and it became necessary to study these points of differences before one felt sure of the interpretation of the results. It is not the purpose of this paper to discuss fully these differences, but a brief summary is necessary for the selection of a physiological preparation which will assay accurately the gastrin samples.

Gastric Fistula Stomach

In this form of stomach we have the most sensitive possible mechanism. The operative procedure is merely to anchor to the abdominal wall, the stomach at a spot where only the terminations of the nerves and blood vessels are to be found. Indeed this very sensitiveness is rather annoying when experiments are first begun upon an animal. Restlessness and fretting apparently set up a spontaneous secretion which may be of appreciable proportions. This factor necessitated several trial runs in the holders, and injections with tap water before the animal adjusted himself to the routine of the experiment.

There was always noted a distinct summation following two successive injections. This may be a true summation of submaximal stimuli, or it may be evidence that there is an inhibitory mechanism to be dealt with as well as a positive secretive one. The following protocol is typical in its illustration of this point.

TABLE I

Dog VIII. Gastric fistula

TIME	QUANTITY	ACIDITY		PEPTIC ACTIVITY	PEPTIC UNITS
		Free	Total		
10	Dressed				
11	3.0 cc.	0.164	0.291		
12	4.5 cc.	0.018	0.237	28.62	85.87
1	6.5 cc.	0.036	0.191	54.76	237.20
1	Injected 1 cc. pyloric gastrin				
2	11.0 cc.	0.164	0.291	72.25	529.7
3	9.5 cc.	0.300	0.446	68.89	436.3
3	Injected 1 cc. pyloric gastrin				
4	36.0 cc.	0.419	0.492	26.01	624.3
5	23.0 cc.	0.410	0.464	28.09	430.7
6	7.5 cc.	0.300	0.382	32.49	162.3

The quantities secured are much larger than in Pawlow dogs, since the entire stomach output is obtained.

The contamination of the juice with saliva must be considered, but it is usually negligible. However, the regurgitation of bile and intestinal content at times does become an important element. The volume is probably not altered to any great extent, but the inhibitory influence of the bile makes accurate pepsin estimations impossible.

Pawlow Accessory Stomach

The operative procedure has inevitably damaged the nerves to a considerable extent. Hence the mechanism should not be so sensitive, and this is borne out by actual experience. Fretting and restlessness rarely complicate the secretory picture. The appetite secretion may perhaps manifest itself once or twice when the animal is first handled, but it is more often absent than present. The stomach can be characterized as "secretorily stable."

Usually summation is either absent or not marked. Hence one injection serves very well to get a measurable comparative response from the stomach. This is shown below.

Dog XI. Pawlow accessory stomach

9.35	Dressed			
10.35	0.96 cc.	Free acid 0.072	Total acid 0.164	
10.35	Injected 1 cc. pyloric gastrin			
11.35	6.6 cc.	Free acid 0.373	Total acid 0.418	Peptic act. 21.16
1.10	1.9 cc.	0.155	0.291	31.36
1.16	Injected 1 cc. pyloric gastrin			
2.16	4.8 cc.	Free acid 0.346	Total acid 0.401	32.49
3.30	1.4 cc.	0.273	0.346	

The quantities are small, but there are no complicating factors of saliva, bile, intestinal contents, and possible faulty drainage, which introduce a mental reservation into one's conclusions.

For these reasons the Pawlow stomach was adopted as the standard for the comparison of gastrin preparations. In cases of very low concentration the gastric fistula stomach gave important supplemental information.

3. Distribution of gastrin in the tissues

(a) Stomach mucosa

Pylorus. There are twenty-six experiments run with injections of pyloric gastrin into Pawlow animals. The protocol of Dog XI previously given is quite typical of the response. The quantities, the hour following the injection, ranged from 5 to 9 cc. In one case 18.0 cc. was obtained. The free acidities varied between 0.35 and 0.47 per cent, with peptic activity of 16 and 25 mm. of albumin. The response of the gastric fistula dogs is shown in Table I. There were ten experiments with acidities and peptic activities corresponding well to those of Pawlow dogs. The quantities of course were much larger.

Fundus. These preparations showed no appreciable difference in activity from those of the pylorus. If anything, they may have been a trifle more active. The experiments comprised fourteen on Pawlow stomachs and one on a gastric fistula animal (see preceding protocol of Dog IV). The following protocol on Dog III shows the response.

Dog III. Pawlow stomach. May 12

9.	Dressed			
10	1.0 cc.	Free acid 0.200	Total acid 0.273	
10.24	Injected 1 cc. Fundus gastrin			
10.54	6.7 cc.	Free acid 0.373	Total acid 0.419	Peptic act. 28.09
11.24	3.1 cc.	0.501	0.547	12.25
11.54	0.5 cc.	0.474	0.547	
12.24	0.5 cc.	0.182	0.364	
1.24	1.0 cc.	0.082	0.145	

Cardia. The cardiac preparations were a trifle less active than those from the other portions of the stomach. However, the difference in activity was so small that it might easily be attributed to slight variations in the method of preparation. In all, there were six experiments upon Pawlow, and ten upon gastric fistula stomachs. The attached protocol is from a Pawlow dog.

Dog III. Pawlow stomach. May 8

8	Dressed			
9	1.0 cc.	Free acid 0.228	Total acid 0.282	
10	1.5 cc.	0.027	0.082	
10.30	Injected 1.0 cc. cardiac gastrin			
11	3.6 cc.	Free acid 0.218	Total acid 0.264	Peptic act. 17.64
11.30	1.5 cc.	0.355	0.419	
1.30	2.3 cc.	0.109	0.200	

It is evident from these experiments that gastrin is uniformly distributed throughout the stomach mucosa, with possibly higher concentrations in the fundus.

(b) Duodenal mucosa

Since secretin has been shown to exist chiefly in the duodenum, it was of interest to us to know accurately the gastrin content, in view of a possible relationship of the two bodies. The first preparation showed a variable activity, which was in no sense comparable to that found in the stomach.

Fearing that some activity had been lost by oxidation in the course of preparation, a second sample was rushed through under exact technique, but it showed no greater activity than the first product. A summary of the experiments run is furnished in the Table below.

TABLE II

Experiments on duodenal mucosa

NO. OF ANIMAL	TYPE OF STOMACH	TOTAL NO. OF EXPS.	SLIGHTLY POSITIVE	FAIRLY POSITIVE	STRONGLY POSITIVE	NEGATIVE
I.....	Pawlow, double vagotomy	7	3	3	0	1
III.....	Pawlow stomach	7	2	5	0	0
XI.....	Pawlow stomach	2	0	2	0	0
VII.....	Gastric fistula	5	1	0	4	0
X.....	Gastric fistula	1	1	0	0	0

Slightly positive.—Slight rise in acid without an appreciable increase in quantity of juice.

Fairly positive.—An unmistakable increase in acid and quantity.

A protocol of Dog III which shows the best secretion by any of the Pawlow animals is appended ("fairly positive").

Dog III. Pawlow Dog. June 30

12.40	Dressed			
1.40	1.0 cc.	Free acid 0.00	Total acid 0.027	
2.40	0.5 cc.			
2.45	Injected 1 cc. duodenal gastrin			
3.45	3.0 cc.	Free acid 0.109	Total acid 0.136	Peptic act. 24.01
4.45	1.5 cc.	0.145	0.218	
5.45	1.2 cc.	0.000	0.063	

While there is a distinct increase in the quantity, yet the acidity rises only slightly. It is not at all of the same order as that following the injection of the stomach preparations.

Below is given a protocol from a gastric fistula dog showing a very pronounced response. It is included for two reasons: first, it will help us in determining the relative concentrations in the duodenum as compared with the oesophagus; second, it is of value in showing how susceptible this type of stomach may be to small stimuli, and therefore, its unreliability as a standard of assay.

Dog VII. Gastric fistula. June 27

10.50	Dressed			
11.50	3.0 cc.	Free acid 0.018	Total acid 0.063	Peptic act. 68.89
12.35	1.0 cc.	Duodenal gastrin		
1.05	12.3 cc.	Free acid 0.337	Total acid 0.355	Peptic act. 33.64
1.35	11.0 cc.	0.419	0.464	21.16
2.35	4.0 cc.	0.109	0.209	
3.35	3.0 cc.	0.000	0.054	132.25

We may conclude that the duodenal preparations give fairly positive gastrin stimulation. The possibility that this is not due to gastrin will be considered later.

(c) Oesophageal mucosa

A tabulation of the experiments run on the oesophageal mucosa will facilitate a comparison with those of the duodenum.

TABLE III

Experiments on the oesophageal mucosa

NO. OF ANIMAL	TYPE OF STOMACH	NO. OF EXPS.	SLIGHTLY POSITIVE	FAIRLY POSITIVE	STRONGLY POSITIVE	NEGATIVE
I.....	Pawlow stomach double vagotomy	2	0	0	0	2
III.....	Pawlow stomach	6	2	0	0	4
VII.....	Gastric fistula	3	0	2	1	0
VIII.....	Gastric fistula	3	0	1	1	1

A protocol of the best response obtained from a Pawlow dog is given. Such a response as this is labelled "slightly positive," chiefly because of the very slight effect on the total acidity, and because the gastric fistula animals have confirmed this activity by much stronger responses. This experiment should be compared with the experiment on the duodenal extract on the same animal.

Dog III. Pawlow dog. July 20

10.30	Dressed			
11.30	2.0 cc.	Free acid 0.000	Total acid 0.054	Peptic act. 10.89
11.43	Injected 1 cc. oesophageal gastrin			
12.43	2.1 cc.	Free acid 0.000	Total acid 0.072	19.36
1.43	1.7 cc.	0.000	0.045	
1.43	Injected 1 cc. oesophageal gastrin			
2.43	2.3 cc.	Free acid 0.000	Total acid 0.072	32.49
3.43	2.7 cc.	0.000	0.054	14.44

An experiment on Dog VII is introduced for comparison with the stimulating effects of the duodenal mucosa on the same animal.

Dog VII. Gastric fistula. July 10

12.20	Dressed			
1.20	0.7 cc.			
2.20	3.5 cc.	Free acid 0.000	Total acid 0.027	Peptic act. 2.25
2.30	Injected 1 cc. oesophageal gastrin			
3.30	2.5 cc.	Free acid 0.000	Total acid 0.054	Peptic act. 3.61
4.30	6.0 cc.	0.000	0.127	
4.35	Injected 1 cc. oesophageal gastrin			
5.35	20.0 cc.	Free acid 0.355	Total acid 0.401	Peptic act. 4.84
6.35	6.0 cc.	0.264	0.319	27.04

If Tables II and III are compared, it will be seen that so far as the Pawlow animals are concerned, the duodenum contains a much higher concentration of gastrin. In truth with no further evidence, one would say that it was absent in the oesophagus. If the two experiments on Dog VII are compared, it will be seen that a second dose of oesophageal gastrin was necessary to provoke a response equal to one obtained by one dose of duodenal mucosa. Gastrin therefore appears to be present in very small quantities in the oesophageal mucosa.

(d) Pancreas

Five experiments were made on four different Pawlow dogs. Of these only one showed evidence of secretion, in which case the quantity of juice was not altered, but the total acidity rose from 0.291 the hour preceding the injection, to 0.364 the second hour after the injection. In the case of the gastric fistula dogs, two experiments on Dogs X and XIII were entirely negative; four others on Dogs VII, VIII and IX were labelled as negative or suspicious. In the latter cases there was a slight increase in volume, associated with very evident quantities of bile. One sample macroscopically appeared to be pure bile. Upon such data we feel justified in concluding that the pancreas furnished no gastrin.

(e) Submaxillary gland

Six experiments on four Pawlow dogs were all negative. Five experiments on three gastric fistula animals were negative, one on a gastric fistula dog with double vagotomy and splanchnics cut was suspicious. The submaxillary gland does not furnish gastrin.

(f) Striated muscle

In three experiments run on two Pawlow dogs, no secretion was obtained. In two experiments on a Pawlow dog (doubly vagotomized) a slight effect is shown as follows. The hour preceding the injection 1.8 cc. of juice was secreted, with free acid 0.246, total acid 0.319. The hour following 2.5 cc. juice, free acid 0.282, total acid 0.328 were obtained. An interpretation of this latter result is not quite clear, since it involves a question of mechanism, a subject which is under investigation. It is sufficient to say that if gastrin be present, it must be in exceedingly minute quantities, otherwise it would have been shown by the normal Pawlow dogs.

(g) Smooth muscle

The muscle from the stomach wall was used for this preparation. Four experiments on four different Pawlow dogs proved negative. One of these negative experiments was made on the animal with double vagotomy, which had previously shown a slight reaction to the striated muscle.

(h) Brain tissue

These experiments are of unusual interest, because they have given us a juice which appears to be other than a true secretion. The tests comprised four experiments on four Pawlow dogs. Two were negative, and two gave a marked increase in volume with no rise in acidity, but with a decrease in pepsin. The protocol attached is illustrative.

Dog XII. Pawlow stomach. August 28

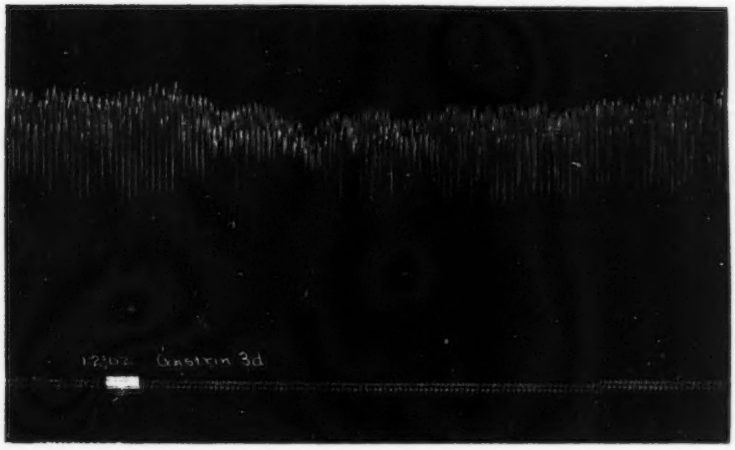
10.35	Dressed			
11.35	1.4 cc.			
12.35	0.6 cc.	Free acid 0.000	Total acid 0.063	Peptic act. 151.29
12.55	Injected brain gastrin			
1.55	3.0 cc.	Free acid 0.000	Total acid 0.018	42.25
2.55	1.6 cc.	0.000	0.018	

This is certainly not a typical gastrin response.

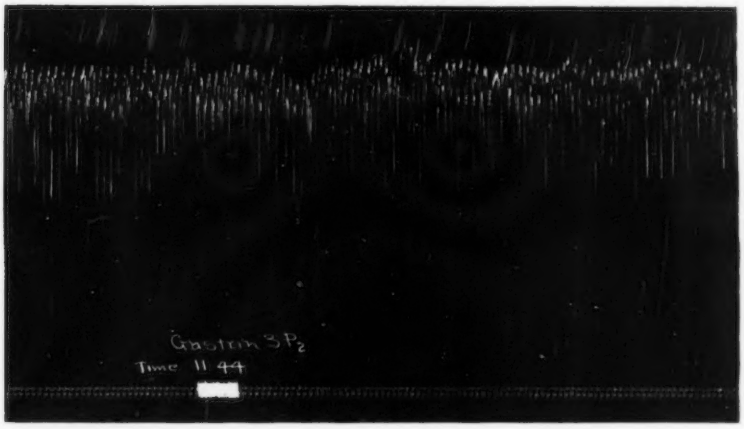
4. Effect of gastrin preparations on blood pressure

It will be recalled that, in making these gastrin preparations, an effort was made at the last to remove the "vasodilatins" with hot absolute alcohol. In view of the oft-repeated contention of Popielski that all of the effects of injections of this class of substances were to be attributed to vasodilation, it became important to know just what were the blood pressure changes under the condition of the experiment. The method of Brooks,[18] of taking the blood pressure without an anaesthetic was adopted. The cannula was introduced into the carotid under ether, from which the animal was allowed to recover completely (usually

[18] Clyde Brooks: Heart, 1910–11, ii, 5.



Gastrin 3d: An active preparation from the cardiac part of the gastric mucosa Time tracing shows seconds.



Gastrin 3P2: The same brain preparation as was used in the previous protocol. Time tracing shows seconds.

one hour is sufficient) before the manometer was attached. The tracing shown below demonstrates that we still have depressor substances present in the preparation. The fall is transient, lasting four to five minutes, but reference to the first protocol (Dog IV) shows that the secretion is at its height thirty minutes after injection. The points of maximum blood pressure depression and maximum secretion do not coincide. It is to be noted that the active gastrin preparation lowers the blood pressure more than the brain preparation. The intravenous injection of our preparations like the secretin preparations of Bayliss and Starling cause a marked temporary fall in blood pressure.

5. Effect of anaesthetics on gastrin stimulation

Following Edkins' method of experimenting, attempts were made in decerebrated cats and etherized dogs, using both intravenous and intramuscular injections, to demonstrate the activity of our preparations. No measurable secretion could be obtained, and we feel that any conclusions based on such a method are open to serious objections.

E. THEORETICAL CONSIDERATIONS AND CONCLUSIONS

1. Nature of the mechanism

A review of the data given above shows that we may consider a one to two hours' secretion of a greater volume of gastric juice together with a higher concentration of acid and an increase in the total output of pepsin as a typical response to an intramuscular injection of gastrin. These results mean that the glands have been stimulated to do work, and this work is not a small, measurable amount, but a quantity of striking magnitude if expressed in physical units.

We feel that Popielski's contention that simple "vasodilatin" action is responsible for this increased secretion is not supported by his, nor by our observations. In the first place, he has not by quantitative methods shown the character of the

responses in different digestive glands after the injection of even crude tissue extracts. Second, although we have not been able to obtain our preparations free from depressor substances by the method recommended by Bayliss and Starling, we have not observed the maximum secretion to take place at the time of maximum fall in blood pressure, but some twenty-five to thirty minutes after the return to normal pressure. Third, we have never observed salivation. Fourth, different tissue extracts exert different effects; the gastric mucosa is most active, the duodenal less so, and the oesophageal only faintly; others are inactive, and brain extract prepared in the same way causes an abnormal secretion, free from acid, lower in pepsin, but greater in volume. Fifth, in two attempts to demonstrate pancreatic juice secretion by means of gastrin, we obtained negative results. Sixth, the indications are that the active ingredient in our gastrin preparations is more stable than the pancreatic secretin of Bayliss and Starling.

As stated above, our preparations, like all the active secretin preparations of Bayliss and Starling or of Dixon and Hamill, were by no means free from blood pressure lowering action, but this does not necessarily call for a general secretion of digestive juices, nor does it follow that the active principle itself, if a true specific secretagogue, causes this fall in blood pressure. As we have a rapid return to normal general blood pressure, but a prolonged secretion, we may consider the action as due possibly to a marked preliminary splanchnic or local vasodilatation followed by a compensatory peripheral constriction; or we may have both a splanchnic vasodilatation as well as a specific stimulation of the gastric glands directly or of the intrinsic nerves of the stomach walls, the actions being due to the same or different substances. Certainly a local vasodilatation is to be expected, and after the primary stimulation of the cells may be an important contributary factor to activity; we have however no experimental data on this point. To be sure our negative results as to other secretions may be due to a more irritable state of the gastric circulation to these general vasodilatins; if we admit this we at once introduce the question of differential

specificity. Again, our negative results with other extracts may in part be due to the lower concentrations of the vasodilatins in these tissues, but we cannot entirely explain the negative results thereby, nor can we so simply explain the abnormal results with brain extract. Two other alternatives occur to us as to these negative results. They may be due to a less complete extraction of the active substances from these tissues since in the preparation of gastrin solutions from gastric mucosa we always have a very much more complete disintegration of the tissue. This disintegration is no doubt due to the digestive action of pepsin in spite of the rapid heating to 90° C. The other alternative is that the peptic digestion of proteins may yield gastrin or substances of similar nature. The abnormal response after brain gastrin may be due to a local or general vasodilatation together with a toxic action on the cells or together with the absence of the specific stimulant for gastric secretion. We admit that in the present state of our knowledge all explanations proposed are necessarily decidedly speculative. We are investigating various phases of these questions, and hope that the purification of the active substance will do much toward cleaning up the same.

2. Distribution of gastrin bodies

It will be well at this point to summarize the findings of others as to the distribution of gastrin activity, in so far as they bear on our results.

Edkins found the activity of the stomach preparations confined to the pylorus. It is sufficient to say that all of his experiments were done under an anaesthetic, that his methods of juice collection were not adapted to the studies of the character of the response, and that his preparations contained a maximum of the vasodilatins (Popielski.)

Eisenhardt worked with juice collected from different portions of the stomach, which was reinjected. The fundus juice was not active, hence his findings do not necessarily controvert our results.

Maydell was unable to find activity in the fundus portion of the stomach. Ehrmann, Tomaszewski, and Emsmann agree with us as to the activity of the fundus portion of the stomach.

Emsmann found the pancreatic extracts active, but not of the same order as the pyloric ones. His experiments were not strictly quantitative, hence his results are border line cases, and their interpretation hinges on the question of what constitutes a positive response.

3. Relation of gastrin to pancreatic secretin

As to the possible relationship of gastrin and secretin, we have no definite experimental data to offer. Maydell and Bayliss and Starling have not found that pancreatic secretin stimulates the gastric juice. The studies of Dixon and Hamill, Bayliss and Starling and others, have shown secretin to be a relatively unstable body. On the other hand, gastrin is quite stable as shown by our experience and that of Tomaszewski, who was able to heat it to 116° for an hour. Until our product is isolated and its effects on pancreatic secretion are established, we cannot state that the duodenal preparations are stimulating the gastric mechanism primarily, or secondarily through the presence of pancreatic juice in the duodenum. The latent period and the character of the response at present incline us to the view that the duodenal extracts are stimulating the stomach primarily and that they merely contain a smaller concentration of the active substance.

4. Specificity of gastrin

The differential distribution of gastrin in the gastro-intestinal tract, and the negative results with tissues of widely varying chemical constitution suggest that it is a specific substance rather than a combination of protein split products.

CONCLUSIONS

1. Evaporation of an acid extract from various tissues leaves a residue, difficultly soluble in 95 to 98 per cent alcohol, which manifests varying degrees of gastrin activity.

2. The gastrin is uniformly distributed throughout the stomach mucosa, is found in much smaller concentrations in the duodenum, and its presence can just be demonstrated in the oesophagus. Preparations of pancreas, submaxillary gland, smooth muscle, and striated muscle are negative.

3. The brain gastrin furnishes an abnormal type of secretion.

4. Pawlow stomachs have been found more satisfactory than gastric fistula stomachs for assaying the preparations. Injections were made intramuscularly.

5. Gastrin in intramuscular doses of 1 cc. (corresponding to 4 to 5 grams of fresh tissue) cause a fall in blood pressure lasting over four to five minutes, a secretion lasting over one and one-half hours, with a maximum between thirty and forty-five minutes following the injection.

6. It is our belief that gastrin causes a true gastric secretion rather than a simple vasodilator response, that it is of a different chemical nature from pancreatic secretin, and that it is a specific substance.

RESEARCHES ON THE EXCHANGE OF ENERGY IN LIVE ANIMAL TISSUES

I. Micro-Calorimetry Applied to Animal Tissues

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Thus far, we believe, the intensity of movement of energy in live animal tissues separated from the organism has been measured by the proportion of exchange of gaseous matter occurring therein. The heat generated by them is measured by respiratory calorimetry. We shall later on discuss the value of the process as applied to this individual case. In this paper we shall attempt to take up the manner in which one may, with ease and accuracy, measure the heat developed by animal tissues separated from the organism, and, at the same time, we shall endeavor to determine the proportion of their exchange of gaseous matter. In doing this, it is our purpose: (1) To ascertain the value of the heat generated by each different animal tissue; (2) To compare the results of indirect respiratory calorimetry, with those obtained by direct means.

MICRO-CALORIMETRY

If a vessel, or receptacle, of perfectly non-conducting material were available, the problem of micro-calorimetry would be reduced to measuring the increase in temperature (x) of the vessel and its contents, during the experiment, and multiplying this increase in temperature by the water-value (V) of the calorimeter and the substance to be investigated. The product (Vx) would exactly represent the amount of heat sought for. Unfortunately, such vessels, or receptacles, do not exist and even the best available allow quite perceptible amounts of heat

to escape. In order to obtain the total value of heat generated it is, therefore, necessary to ascertain such loss, and to add it to the expression (Vx) representing the heat retained by the calorimeter. Let us take any vessel or receptacle; the amount of heat which it allows to escape within a specified time will depend on the nature and thickness of its walls, on the condition of its surface, the environment in which it is located, and the difference (T–T_o) between the temperature of the vessel (T) and the outside temperature (T_o); therefore, in the case of an identical vessel, placed in the same environment, the amount of heat lost will depend only on (T–T_o). Experience shows that within the limits of the temperatures used for physiological researches there is a perfect proportion between the amount of heat lost by conduction (Q) and the difference (T–T_o) between the temperature of the vessel and the outside temperature. Therefore, we find,

$$\frac{\zeta}{(T - T_0)} = \text{constant}$$

It is this peculiar feature of the calorimetric vessels, which makes it possible to ascertain the heat lost by conduction. This constant was adopted, after proper trial, by Rubner (1) and Hill (2) the authors of the only two methods of micro-calorimetry which, to the best of our knowledge, exist in physiology. Rubner adopted a constant (K) representing the amount of heat that a calorimetric vessel allows to escape during one hour (T–T_o) being equal to 1°C. Hill adopted a constant (k) representing loss of temperature, the connection between same and the constant K (loss of heat) being represented by the relation:

$$k = \frac{K}{\text{water-value of the calorimeter and its contents}}$$

Let us now suppose that during an experiment the average value of (T–T_o) is equal to (m) degrees. The heat loss will be equal to (mK). In actual practice, however, how can we determine the average value of (T–T_o) during an experiment? According to Rubner and Hill, it will not be possible to establish this average value of (T–T_o) whenever the outside temperature (T_o)

should happen to oscillate arbitrarily. It is, therefore, absolutely necessary to annul, or counter-balance, the variations of the outside temperature. Rubner succeeded in obtaining this by placing the calorimetric vessel in an incubator the temperature of which was maintained absolutely stable by a complicated and expensive process.

Hill preferred to annul, or counterbalance the variations of (T_o) by connecting the calorimeter used in the experiment to another controlling vessel, in accordance with the differential method. He then demonstrated that, if k and k', coefficients of loss of temperature of these vessels, are equal to each other, it is possible to ascertain the total amount of heat generated, given the value of (T–T') during the experiment, the difference between the respective temperature of the two calorimetric vessels being obtained directly by the mere reading of a galvanometer connected to two thermo-electric solders, placed respectively one in each of the calorimetric vessels.

Rubner's method requires a difficult installation in order to be carried out in practice, and presents no advantages of precision or convenience over that of Hill. Hill's method, according to the author, has the great advantage of not requiring continual readings of the difference of temperature (T–T'), that such readings need be made only every hour or even every two hours, during the longer tests. This fact is a consequence of the regularity with which (T–T') oscillates, all irregular variations of the outside temperature having been annulled as we have seen. This advantage is not, however, peculiar to the method, inasmuch as (T–T') depends also of the manner in which the heat is developed within the calorimeter. It suffices, for instance, that heat be generated in an irregular manner, for (T–T') also to oscillate irregularly, thereby rendering absolutely necessary the continual reading of the differences of temperature (T–T').

Furthermore k and k' always differ in practice by a perceptible amount which varies at each experiment. To this is perhaps due the fact that his method shows in trial tests on an average, errors of 4.8 per cent and 5.3 per cent. If we take the extreme errors (-7.7 per cent and 9 per cent) of a series,

we find, on comparing the two experiments, that the error may amount to 16.7 per cent. If we take the extreme differences of the series which the author states was carried out with more care (series of 3 tests, average error = 3.2 per cent, extreme errors = −3.9 per cent and 3.8 per cent) we shall find 7.7 per cent. The amplitude of these errors is excessive for the problem which we purpose solving. Moreover, Hill's method requires the use of the thermo-electric process to obtain the differences of temperature (T–T') and, the use of a good galvanometer, which cannot always be found in all laboratories.

We shall now proceed to describe the method used by us and which, although relatively elaborate, is as precise as may be desired, and requires so simple an outfit as to be within the reach of any investigator. The solutions of Rubner and Hill are, as we have just seen, but the direct consequence of their belief that it is only possible to calculate the losses in heat by conduction, or else when the outside temperature does not oscillate or, again, when such oscillations are annulled (counter-balanced) by the differential method. Their belief does not, however, strictly correspond to the actual facts. Let us imagine a calorimetric vessel having in its interior one of the solders of a thermo-electric battery, the other solder being placed outside the calorimeter. The readings of the galvanometer will show the difference (T–T_o) between the temperature of the calorimeter (T) and that of the outside (T_o). Based on the frequent readings taken, let us make a record of the differences (T–T_o) on chart paper, this curve showing the time at the axis of x and the differences (T–T_o) as ordinates. As the calorimetric vessel admits of a constant (K) of loss in heat, it is necessary to

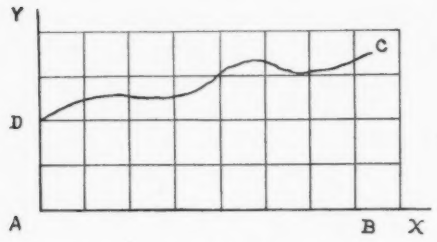

Fig. 1

find out, in order to ascertain the heat lost by conduction, the average difference of $(T-T_o)$ for the length of time during which the experiment was conducted. The problem, therefore, is reduced (as shown in fig. 1) to determine the average ordinate of the curve $(T-T_o)$.

It is evident that the area of the surface $ABCDA$ is equal to the base AB multiplied by the average height. AB being known, the area $ABCDA$ can easily be determined (as closely as desired) by the usual different methods of squaring. The unknown term, the average distance from curve $(T-T_o)$ to base AB, is, therefore, equal to the area of the surface $ABCDA$ divided by AB.

In other words, $ABCDA = s$ and $AB = t$, or the duration of the test.

We therefore have: Average value of $(T-T_o) = \frac{s}{t}$.

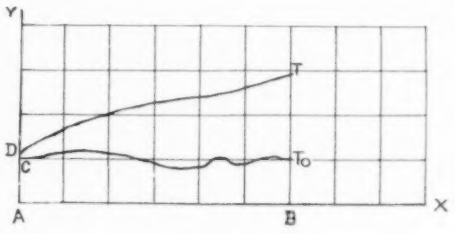

Fig. 2

Let us now suppose that we have no galvanometer to obtain directly the difference $(T-T_o)$. It will then suffice to place a thermometer in the calorimeter and with another thermometer follow the oscillations in the outside temperature. By charting these readings to two coördinate axes we shall obtain the two curves shown in the figure 2. By similar reasoning, it will be seen that the average difference of temperature $(T-T_o)$ is equal to the surface CT_oTDC divided by its projection over the axis of x (that is by AB) time of duration of the experiment. These are the principles on which we have sought to ascertain the heat generated, and which we may subdivide into two parts:

The first, which is retained by the calorimeter, is equal to the water-value (V) of the calorimeter and of the matter which is being investigated, multiplied by the differences of temperatures inside the calorimeter, from the time the experiment is started

until its conclusion ($= x$); the second part that which is lost by conduction, is equal, as we have seen, to $K\frac{s}{t}$.

The total amount of heat (Q) is therefore represented by the expression

$$Q = Vx + K\frac{s}{t}$$

A FEW PARTICULARS OF OUR METHOD OF MICRO-CALORIMETRY

Our calorimeter is, like Hill's, the Dewar flask, i.e., a vessel or receptacle, with silver plated double glass walls, separated by vacuum, such as the bottle generally and commercially known as "Thermos." Its mouth or opening is closed by a rubber stopper. This stopper has the following devices: (1) A very precise and sensitive thermometer, previously verified, and capable of recording up to one hundredth of a degree centigrade. The mercury reservoir of this thermometer reaches down in the vessel, almost touching the bottom thereof; (2) Two fine tubes, to which are adapted rubber tubes which extend outside the vessel, the extremities of these rubber tubes being closed and tied with thread during the experiments. It is to be noted that the glass tubes proper fit exactly into the stopper, and do not project beyond same, and they are used for the purpose of collecting the gases from the calorimeter, for later analysis.

To determine the water-value of the calorimetric vessel, the following method is adopted:

A certain quantity (P) of water is placed inside the vessel, and after a certain time has been allowed to lapse, the temperature (T) of the vessel and of the water is taken; a further amount of water (p) is then added of the temperature (t) and a reading is taken of the final temperature (t'). Let (x) be the water-value of the calorimetric vessel; (t') is equal to:

$$t' = \frac{xT + PT + pt}{x + P + p} \therefore \quad x = \frac{t'(P + p) - (PT + pt)}{T - t'}$$

By repeating this procedure several times, adding water now warmer than that in the calorimeter, now colder, one is able to

determine very closely the constant of the apparatus. It is a mere matter of care and practice.

Constant K representing the loss in heat, is determined in the following manner: In the calorimeter, the water-value of which is already known, a weight (P) of water is poured, at a temperature (T), and the apparatus and its contents are then allowed to cool. Frequent readings of the inside temperature are taken as the process of cooling progresses, and, at the same time, record is kept of the outside temperature. These two series of readings are transcribed to chart paper, on which reference is likewise made of the time. The average value of $(T-T_o)$ is determined by the methods described above.

Let us suppose that the vessel became cooler by x degrees. The amount of heat that is lost by conduction, for 1° of difference, is equal to

$$\frac{\text{(water-value of the calorim. and its contents)} \times x}{\text{average value of } (T-T_o)}$$

This result is reduced to 1 hour by a simple proportion. The tests we made, in connection with the proportion of heat lost in relation to the difference in temperature $(T-T_o)$, namely, the verification of the existence of a constant K, consisted in allowing the calorimeter to cool, a given or specific amount of water having been placed in it, due record being made at the same time of the variations in the outside temperature, so as to ascertain the average value of $(T-T_o)$. These experiments, or tests, although carried out according to different technical procedures, confirm those made by Rubner and Hill, with but one restriction: the constants k or K vary slightly in accordance with the environment (air or water, for instance) in which the calorimeter is placed. It is, therefore, evidently necessary always to place the calorimeter in the same environment, whenever a precise experiment is desired.

PREPARATION OF THE ANIMAL TISSUES FOR EXPERIMENTS OF MICRO-CALORIMETRY

We have, in a measure adopted the technical process of Battelli-Stern (3), which, however, we have endeavored to improve in order that it might the better answer our purpose. In the experiments carried out, we have always made use of tissues taken from a dog immediately after its death by bleeding. The organ (the liver, for instance), is quickly cut into pieces, and finely ground thereafter in Lattapie's apparatus. The pulp thus obtained is placed in a capsule of a specific tare, so as to attain 50 grams. This pulp is then suspended in a fluid which, for the sake of convenience we shall call artificial blood, and which is obtained in the following manner: 125 grams of blood of the dog whose organ is to be experimented upon are placed into an Erlenmayer vessel or receptacle, containing small glass balls (pearls); it is then shaken, filtered through a cloth to separate the fibrin, centrifugated to separate the red globules; the serum is then decanted. The original volume is reëstablished by the addition of a simplified formula of Locke's fluid as follows:

NaCL.....	9.0 grs.	$CaCl_2$.....	0.20 centgrs.
KCL.....	0.20 centgrs.	Water.....	1000.0 grs.

The whole is again centrifugated, and the preceding operation is repeated retaining the previous volume of 125 grams. Thus, one will have obtained a suspension of red globules in Locke's simplified fluid. The purport of all this is merely to eliminate the blood serum and, with it, the salts capable of collecting carbonic acids. Indeed, when this precaution is not taken, the proportion of CO_2 and the respiratory quotient of Pflüger are likely to vary much in similar experiments, while, if the course we have outlined is followed, this will be avoided. For the same reason, we simplified Locke's fluid, eliminating the bicarbonata. The respiration of the various tissues occurs, under the circumstances with great vigor and to the satisfaction of the investigator.

HOW TO CONDUCT THE EXPERIMENTS

The tissues suspended in the artificial blood is placed in a glass jar of about 1 liter capacity, which is then stoppered and submerged in the water of an incubator the temperature of which should be more or less the same as that at which the experiment will be made. Our incubator consists of a zinc tank with about 60 liters of water. The temperature is maintained by gas burners connected to an ordinary regulator. In this tank should also be placed the calorimeter unstoppered, as well as its rubber stopper in which, as already explained above, a thermometer is inserted. Every now and then the water which enters the Thermos (calorimeter) is emptied out and the receptacle is allowed to replenish. By this procedure, and exercising particular care, the same temperature is obtained for the calorimeter, its stopper and the tissue on which the experiment is to be made. This method also has the advantage of saturating the red globules of the artificial blood with oxygen. The calorimeter is emptied out the last time, without removing it from the incubator, the emulsion of tissues is poured into it and the receptacle is closed with the stopper fitted with the thermometer. The calorimeter is then attached to a shaker consisting of a wooden board plunged into the water of the incubator. This board is connected, on its other side by means of a suitable device, to an electric motor which will cause it to move back and forth. Thus the double result is obtained: (1) shaking in a thorough and uniform manner the emulsion in contact with the air obtained in the calorimeter, which facilitates its respiratory exchange, and (2) rendering homogeneous the temperature of the water in the incubator, which is most convenient for ascertaining the exact outside temperature (T_o). A good thermometer, similar in all respects to that which is fitted to the stopper of the calorimeter, is dipped into the water of the incubator. When the experiment is concluded, the calorimeter is immediately removed from the incubator and the gases, which are necessary for subsequent analysis, are collected from the glass tubes fitted into the stopper of the calorimeter, as already explained.

REMARKS

The calculation of the total heat generated is represented by the formula

$$\zeta = Vx + K\frac{s}{t}$$

For the first part, thereof, it is always very hard to ascertain the value of the specific heat of the material under examination and it is therefore generally considered as equal to that of water. This may, however, mean a very perceptible error. Inasmuch as the second part is not subject to the same mistake, and is obtained with much more accuracy it is often preferable to increase considerably the difference $(T–T_o)$ between the temperature of the calorimeter and that of the outside environment, by allowing the latter to become cooler. Under the circumstances, the expression $K\frac{s}{t}$ increases at the expense of the expression Vx which may be annulled or rendered negative. The oscillations of the temperature of the incubator, which is equipped with an ordinary regulator are very slow and this makes it easier for the investigator. As a rule, our experiments are conducted with the calorimeter completely submerged in the water of the incubator; the incubator, however, is not really indispensable, and the experiments may be conducted in the open air.

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